

nature

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Back to the freeze?

A COUPLE of years ago (January 10, 1975) we reported on an extremely gloomy set of figures which showed how openings for senior university posts in the United Kingdom had all but disappeared in 1974 as a result of a general freeze in hiring. We have now brought the figures up to date by analysing advertisements for the years 1975 and 1976. Things are, at least temporarily, much better.

In the past two years the level of hiring seems to have been back fairly close to the levels of the late 1960s and early 1970s. Of course this indicates that the backlog has not been, and is never likely to be cleared. Some posts have disappeared for ever. But it may help to shake loose, in the course of time, the rigidity with which the universities were beginning to be afflicted. That is, unless the next rather ominous cloud on the horizon can be dispersed.

That cloud is the recent warning by the University Grants Committee that next year's (1977-8) grant to universities for salaries, maintenance and capital expenditure could be as much as 4% down in real terms. This figure first

appeared in mid-1976 when the raising of student fees was seen by the Department of Education and Science not only as a way of bringing in more revenue to universities but also as a restraint on growth in student numbers and therefore in university financial needs. Since then there has been much re-adjustment, including the recent public expenditure cuts, but the result of it all is that we are back to the figure of 4% as the basis for discussion and warning.

Three quarters of all UGC disbursements go on salaries and so the next round of the national pay policy, not yet openly discussed, could make the figure look disastrous—or even quite tolerable; such is the uncertainty in which universities, once used to quinquennial planning, now live. But whatever happens there is a limit to the amount that can be saved by not painting, not cleaning and not heating. Very soon we could be back to solving the universities' financial problem by again venturing on the capricious course of not hiring. □

Amaze, test, shock your friends

THE festive season permits us to see ourselves as others see us and no better than through the scientific books, toys and kits showered on our children—and ourselves.

Here we have a book in which it says that if you drop a slice of buttered bread from a height it will land more often with its buttered side down. This is because the layer of butter makes one side heavier so "there is a tendency for the butter-side to fall faster". Yet further on we are told that in the absence of air "heavy things and light ones get up speed with the same degree of acceleration". And to complete the confusion we hear about a lorry with a load of canaries. When the birds all fly up in their cages "the weight on the tyres is reduced".

Or here is an excellent planisphere to show the position of the stars. The stars, it says on the back "appear to move in the same way as the sun" except that the "apparent movement of constellations near Polaris is more limited". And your absolute beginner, venturing out just after Christmas in the early evening might wonder why a couple of the most prominent 'stars' in the sky don't rate a mention.

They do get mentioned elsewhere in an encyclopedia for boys and girls. "The Universe is made up of the Earth we live on, the Sun and its other planets, thousands of other small bodies—and space itself . . . a planet is a big ball of rock and gas".

Nothing about astronomy in the Super Quiz Book for Girls; the question on stars runs "where were Paul

Newman and Omar Sharif born?" In the same book there is a picture of a lady in a white coat which bears the label 'UKAEA'. A machine is applying lipstick by remote control—"Of course, the machine was developed for handling radioactive materials, but something like it may well one day be in every home". The same quiz book also offers intelligence tests—"use these tests to see how much quicker and brighter you are becoming, how you compare with your friends".

Home experiments seem to have become simpler and certainly safer with the years. No longer do you "hold the two handles of the induction coil and gradually insert the steel rod until you can no longer bear the tingling sensation. Compare your performance with that of your friends". But even a book of delightfully simple experiments has an oddity or two. Place a bottle between your mouth and a lighted candle. Why does the flame go out when you blow? "The air moving past the bottle creates a partial vacuum behind the bottle, and the air rushing in to fill this vacuum puts out the flame".

After junior science, the children can at least retreat into the unambiguous world of their first knitting kit—"Place a needle under your left arm. Hold the end of the yarn tightly in your left hand. Take the yarn coming from the ball into your right hand between your thumb and middle finger. Turn your index under the yarn between your hands towards you and point your index forward. There is a loop on your index". □



Articulating the aims of science

Nicholas Maxwell, of the Department of History and Philosophy of Science at University College, London, offers this comment on the current state of the art

MODERN science is, I suggest, seriously harmed by a widespread attempt to make science conform to a wholly inadequate philosophy of science—a philosophy of science which quite grotesquely misrepresents the basic intellectual aims of science.

This inadequate philosophy of science may be called 'standard empiricism'. It is carelessly and unthinkingly taken for granted by the scientific establishment today. It is defended by almost all contemporary philosophers of science—from Popper to Kuhn, Lakatos, Hesse and Grünbaum—as constituting a rigorous, rational conception of science, even though elementary arguments show decisively that the view fails miserably to exhibit science as a rational enterprise.

The basic idea of this philosophy of standard empiricism is that the fundamental intellectual aim of science is simply to improve our knowledge of value-neutral factual truth, scientific progress being assessed in terms of how successfully this aim is being realised. *A priori* knowledge about matters of fact being denied, it is held that in science theories must be assessed, in the end, solely in terms of empirical success.

In fact, of course, science does not seek to improve our knowledge of factual truth as such; rather, quite properly, science seeks to improve our knowledge of *important* factual truth, truth that we deem to be in some way valuable, significant, interesting, beautiful, useful, from either a cultural or a practical, technological standpoint. Progress in science is assessed in terms of the amount of valuable factual truth that is being discovered: accumulation of trivia, however extensive, does not, and ought not, to be judged to amount to progress.

Once it is acknowledged that a basic intellectual aim of science is to discover valuable truth, it becomes clear that the aims of science must remain permanently and profoundly problematic. What is there to discover that is, in one way or another, valuable or useful? In what direction do really important, exciting future discoveries lie? What ought to be the general priorities of scientific research? What is at present of greatest importance or value,

from a human, social standpoint? Important to whom? What kinds of things will we most need to know in fifty or one hundred years' time? These questions are all highly controversial and problematic just because they concern the domain of our ignorance, and difficult issues concerning human needs, aspirations and values.

It is of fundamental importance that we make the best possible choice of aims for science; and it is almost inevitable that we will fail. Here above all, then, we need to proceed intelligently, critically, imaginatively, wisely. If science is to proceed in a truly rigorous and rational fashion it is essential that we articulate, explore and criticise possible and actual aims for science as an integral part of scientific enquiry, providing ourselves with a wealth of critically examined alternatives, so that we may enhance our capacity to choose wisely and well. Quite generally one can say that rigour involves making explicit and so criticisable that which is implicit, influential and problematic. The best aims for science lie in the direction of the overlap between that which is scientifically realisable, and that which is humanly desirable. In order to give ourselves the maximum chances of discovering this problematic region of overlap we need to articulate both that which we conjecture to be scientifically realisable and that which we conjecture to be humanly desirable.

A truly rigorous, rational science would, then, include three kinds of contributions to science. In addition to contributions at the level of experiment and theory, we need to include contributions which articulate, explore and criticise possible aims for science. Scientific journals, textbooks, and educational courses need to include a discussion of all three kinds of contributions. It ought to be possible to win a Nobel prize by a sufficiently brilliant contribution at any of these three levels. We need to regard our scientific knowledge as consisting not only of what we know, but also of our best conjectures about what we do not know, but hope and desire to discover. We are never completely ignorant of that which we do not know; for if we were, we could have no basis for our

assumption that the domain of our ignorance will not suddenly intrude upon the domain of our knowledge in some violent, unruly fashion, entirely disrupting the straightforward predictions of our present day scientific theories. Without some vague knowledge of that of which we are ignorant, all scientific knowledge would be impossible.

All this was well known to Einstein (*Ideas and Opinions*, Souvenir Press, 1973). He understood clearly that theoretical physics is only a worthwhile, rational enterprise when pursued as an attempt to discover a conjectured unified harmony in nature, the basic aims of physics thus being permanently and profoundly problematic. The success of Einstein's contribution to physics arose in large measure from his ceaseless concern to articulate and develop the best possible aims for physics, taking into account both existing knowledge and our desire to discover unity and harmony, appropriate heuristic rules then being formulated as guidelines to the development of new theories. I suggest that this kind of aim-oriented empiricist methodology, exploited with such success by Einstein within the restricted field of theoretical physics, is of universal relevance to science and technology.

At present aim articulation, as envisaged here, does not proceed as an integral part of scientific enquiry, just because most scientists take standard empiricism for granted. Once standard empiricism is accepted, scientific rigour and rationality actually seem to require that articulation and discussion of possible and actual aims for science be excluded from the intellectual domain of science. Thus, as a result of the attempt to make science conform to the intellectual ideals of standard empiricism, scientists have served to undermine the rigour and rationality of science, precisely because the vital task of articulating and exploring possible aims for science has been somewhat inhibited and repressed. As I have argued in greater detail elsewhere (*Phil. Sci.*, **41**, 123–53, 247–95 (1974); *What's Wrong with Science?*, Bran's Head Books, London, 1976), there is at present an urgent need for the scientific community to free science from the irrational ideals of standard empiricism, and to put into practice the kind of aim-oriented empiricist conception of science so successfully practised by Einstein. □

Gemona: the reckoning

An earthquake struck Gemona di Friuli, Italy, last May. Nick Ambraseys assesses its impact

THE Gemona earthquake of May 6, 1976, affected a large region in North-East Italy which, during the past nine centuries, has been devastated by a series of large shocks that have had recurrence intervals of almost total quiescence of 150 to 200 years. The largest of these events occurred on January 25, 1348, and on March 26, 1511. These earthquakes were associated with radii of perceptibility of 650 to 750 km and with aftershock sequences that lasted from three to six months. In addition, the shock of 1348 triggered a slide in the Gail valley, the volume of which exceeded 1,000 million m³, a volume about four times larger than that triggered in 1963 at Vajont by the rapid draw-down of the reservoir level.

The Gemona earthquake occurred at 2100 hours local time near 46.24°N 13.28°E and had a focal depth of less than 10 kilometres; it had a surface-wave magnitude of 6.3 and was preceded by a single foreshock of magnitude 4.5. Another phenomenon which could have been premonitory was the appearance of a glow in the sky above the northern part of the epicentral region which persisted for some time after the earthquake.

There is no evidence that the earthquake was associated with surface faulting. Ground deformations originally suspected to be of tectonic origin in Montenars, Sedilis, Susans and Avasinis proved on examination to be of secondary nature. Persistent patterns of ground fracturing caused by slumping do not align with any minor or major fault.

The earthquake produced ground fracturing on flat and sloping ground, as well as a few cases of very local liquefaction. Landslides, rockfalls and debris-flows from steep slopes triggered by the shocks and heavy rains caused serious damage to property. Most of the rock faces and slopes that collapsed were already unstable.

More than three-quarters of the houses in the region are very old. Their walls consisted of coarse, short-bedded, badly-laid rubble masonry, sometimes mixed with bricks and with great thicknesses of lime or clay mortar joints, concealed by plaster or rough cast. Floors and roofs are heavy, resting on joists of timber. The use of tie-rods in masonry walls and laid at floor and roof level has saved many houses from collapse. New houses, mostly those built with some care after the local shocks of 1928 and 1939,

have brick walls and reinforced concrete columns with cast-in-situ slabs. These houses, as well as structures built more recently with few openings and light roofs, survived the shock with comparatively little damage.

Both old and new houses which had recently been renovated suffered the most. A common cause of unintentional weakening was found to be due to the installation of electricity and water conduits in old houses, the re-location of bathrooms and sewer and drainage pipes in old walls, and the addition of extra rooms and floors in old dwellings. Damage to only less than a quarter of the houses in the region could have been prevented had modern houses been built to resist earthquakes; the rest were built long before the advent of modern technology. Lack of ductility and eccentric stiff structural elements in open ground floors were the main cause of damage to modern reinforced concrete houses and blocks of flats. Prefabricated constructions particularly in the industrial areas suffered more than other types of structures. The affected region was not considered to be highly seismic and very few buildings had been designed and built to resist lateral forces.

Within the region of maximum damage, intensities varied erratically from place to place and in only a few isolated points did they reach an intensity IX on the Mercalli scale. In this region the distribution of damage was influenced more by the type of structures shaken than by their foundation conditions. Intensities underground were much smaller than those experienced on the surface. In deep

caves, delicately poised rocks, thin stalactites and stalagmites showed no signs of disturbance.

The maximum horizontal ground acceleration deduced from displaced objects in the meizoseismal region reached values of 75%g associated with very high frequencies. The peak acceleration of the main shock recorded at a focal distance of about 30 kilometres (S—Start time=3.8 sec.) was 37%g and the velocity 32 cm sec⁻¹. Peak accelerations of aftershocks of magnitude 5, recorded at smaller distances, reached values in excess of 50%g.

The main shock killed 965 and injured 2,286 people. The average rate of fatalities for the whole area is just under 1% of the affected population. All age groups except the infants and elderly suffered fatalities roughly proportional to their numbers. In densely populated villages and in historical town centres the more agile group of youths suffered more casualties in their attempts to seek refuge outdoors into narrow streets where they were crushed under falling walls.

The damage caused by the main shock was estimated late in May at \$1,707 million. Damage to dwellings was \$573 million, to agriculture \$488 million, to industry \$244 million, handicraft industry \$146 million, public buildings \$122 million, commerce \$110 million, roads \$16 million and aqueducts \$8 million.

Preliminary estimates today for the total damage caused by the main shock and its aftershocks including loss of revenue from commerce amounts to \$2,859 million. The total loss to buildings which includes 237,000 rooms of houses which were either totally destroyed or seriously damaged (excluding contents) has been estimated at \$1,078 million. This figure is based partly on August–September 1976 market replacement values and partly on assessed



Destruction at Friuli

values. It includes replacement cost for 1,650 hospital beds and for seriously damaged or destroyed public buildings. Damage to industry, including handicraft and small industry is estimated at \$599 million. Damage to agriculture amounted to \$479 million which has now risen to \$610 million.

Damage to public works and utilities amounted to \$240 million including repair costs for the water supply, sewers, electrical facilities, aqueducts, road and railway communications. Loss of revenue from commerce is estimated at \$240 million, and \$176 million will be required for the repair and extension of the flood control of the Tagliamento valley. There is \$49 million-worth of damage to school buildings and a

sum of \$96 million has been allocated for the repair of the few historical monuments that survived the shock. About 600 churches were destroyed or damaged beyond repair and damage to historical buildings, castles and works of art cannot be calculated.

One of the conclusions that can be drawn from the study of the Gemona earthquake is that this event could easily have been expected from what we know about the seismicity of the region. This earthquake demonstrated once more the economic vulnerability of developed and densely populated regions of apparently low seismicity to strong earthquakes of long return period but short recurrence interval. It also demonstrated the need for the

application in such regions of minimum earthquake resistant design requirements in the construction of new buildings and for the strengthening of old ones, particularly of historical monuments and art treasures. The amount and extent of damage caused by the earthquake and by its after-shocks have important implications for the insurance industry and for the social and economic planning in earthquake areas. □

The field work reported in this study constitutes part of the work carried out on a UNESCO Earthquake Reconnaissance Mission to Friuli (Ambraseys N. "The Gemona di Friuli (Italy) earthquake of May 1976". UNESCO Rec. Mission Report, No. 273.182 (1976). UNESCO, Paris). The writer would like to record his indebtedness to the President of the Regione, Dott. A. Comelli, the Director of Forestry, Dott. R. Querini and to the Vigili del Fuoco for their generous cooperation.

Prediction: the negative aspects

The ability to predict earthquakes should reduce deaths, injuries, and property damage, but the prediction itself can have some negative effects. Henry Lansford sent this report from Boulder, Colorado, on a new study of the socioeconomic impacts of earthquake prediction:

WHEN social scientists go out into the real world to study a subject that has attracted a good deal of public attention, they usually come up with two classes of conclusions. The first class includes unsurprising findings that often provoke sceptics to snort that social science research is a waste of time—"common sense would have told you *that!*" But in addition to confirming some things that common sense might suggest, good social science research can turn up some surprises too—the unexpected insights into human behaviour that the sociologists like to call "counter-intuitive conclusions."

An 18-month study* of the potential impacts of earthquake prediction in the United States, conducted at the University of Colorado with support from the National Science Foundation, has produced both kinds of conclusions. The research team concluded, for example, that although the first credible predictions of damaging earthquakes months or years before they occur can save many lives and reduce property losses, such predictions can also result in severe economic depression and social disruption in the communities for which earthquakes are predicted. Although nobody had given much thought to this negative aspect of earthquake prediction, it should not come as a great surprise that property values and tax revenues will decline in an area for which an

earthquake is predicted; that business activity, employment opportunities, and public services will be reduced; and that the sale of earthquake insurance policies will cease as soon as the prediction is issued.

But there are also some counter-intuitive conclusions. The researchers found few indications that homeowners who have small equities in heavily mortgaged homes would simply walk away and stop making their monthly payments, contrary to what common sense with a touch of cynicism might suggest. They found that savings deposits in banks in the "target community" for which the earthquake is predicted would go up instead of down with the rest of the economy, as people save money instead of spending it on household appliances or other property that might be damaged or destroyed by the earthquake. And, perhaps most surprising of all, they found that 40% of the residents would remain, rather than evacuating either temporarily or permanently, in an area for which an earthquake five times as great as the one that devastated San Fernando, California, in 1971 has been predicted, even if they believe that the prediction is accurate.

The research team, which included both physical and social scientists, did most of their field work in California, which has had more recent experience with earthquakes, as well as more trepidation about future ones, than any other part of the United States. The team also looked closely at earthquake-prediction research that has been going on in China, Japan, and the Soviet Union. They began by getting estimates from seismologists of the probable characteristics of the first official, scientifically-based earthquake prediction

in the United States and of the way in which it would probably be released to the public. Then they formulated two "prototype" predictions: one assumed a five-month lead time, a 50% probability of occurrence, and an earthquake with a magnitude of 6.3 on the Richter scale; the second assumed a three-year lead time and a 7.3 magnitude. Then they conducted more than 1,000 interviews throughout California, with federal, state, and local government officials, executives of large and small businesses, managers of large and small news media and individual families.

Three of the study's findings are regarded as particularly significant:

- Under existing laws, it is not clear whether federal disaster-relief funds can be committed on the basis of an official earthquake prediction or whether it will be necessary to wait until earthquake damage has occurred before committing funds that might have helped prevent the damage.
- No workable alternative to earthquake insurance exists for indemnifying those whose property is damaged or destroyed by an earthquake, although the California state insurance commissioner has stated that he will stop the sale of new earthquake insurance policies in any area for which a credible official earthquake prediction has been issued.
- Damage estimate maps, based on solid scientific data and published by news media in the threatened area, should reduce property damage considerably if the earthquake prediction provides enough lead time to reinforce structures and undertake other damage-prevention measures. □

* J. Eugene Haas and Dennis S. Mileti, *Socioeconomic Impact of Earthquake Prediction* (Institute of Behavioral Science, University of Colorado, Boulder, Colorado; 1976).

USSR

What sort of turning point?

Vera Rich reports on the fate of dissident Soviet scientists in light of the recent release of Vladimir Bukovskii.

THE release of Vladimir Bukovskii, in "exchange" for the Chilean Communist leader Luis Corvalan, brings to the West one of the most significant figures in the human rights movement in the Soviet Union. It does not, however, appear to indicate any general amelioration of the plight of dissidents; indeed, following Bukovskii's release, a number of other dissidents and refuseniks, including several prominent scientists, were arrested.

Bukovskii, who was first arrested in 1963 while still a student of biology, has spent ten of the last thirteen years in penal psychiatric hospitals, prisons, and strict regime labour camps. During one of his brief periods of freedom he managed to inform the outside world of the growing Soviet practice of employing psychiatric methods to repress political dissidents, thereby posing an ethical problem to Western psychiatrists as to what should be their professional relationship (if any) with those of their Soviet colleagues known to be involved in this abuse. Imprisoned once again, in 1974 Bukovskii was sent to the strict regime labour camp at Perm', where with his fellow prisoner, the psychiatrist Semeon Gluzman, he wrote the now-famous *Manual of Psychiatry for Dissidents*, which sets out self-defence strategies for those whose political or ethical convictions are judged by the authorities to be evidence of insanity.

Immediately on his arrival in Zurich, Bukovskii thanked those who had campaigned for his release and announced his intention of devoting the rest of his life, if necessary, to campaigning for his fellow prisoners-of-conscience. In the euphoria of release, he said that he felt his liberation marked an important new trend in Soviet policy and was "a great victory for everyone". This hope, however, does not appear to have been justified. The "exchange" procedure which brought Bukovskii freedom has been tacitly denied by the Soviet authorities; officially, Corvalan was released due to the efforts of "progressive forces" throughout the world, while Bukovskii was expelled from the Soviet Union as a terrorist. And, a few days after Bukovskii's release, another former long-term psychiatric detainee, the electrical engineer Vladimir

Borisov, was rearrested.

Borisov, who was released in 1974, had spent the major part of the previous eleven years in penal psychiatric hospitals for having organised an unauthorised youth group. During his captivity, he took part with Viktor Fainberg in a hunger-strike to protest against the conditions under which the prisoners are kept and the denial of their basic rights. Borisov's rearrest, on December 25, was allegedly in connection with the anti-Soviet slogans which appeared on walls in Leningrad earlier this year; dissident sources in Leningrad, however, interpret it as a warning from the authorities not to assume that Bukovskii's release marks a new 'thaw'. Borisov is at present, confined in a Leningrad psychiatric hospital in a block for 'acute' and violent cases, and has not yet been seen by the 'commission' of three psychiatrists which, legally, must decide on his case. His wife Irina is demanding that the commission should consist of psychiatrists whom she can trust, and has asked for Semeon Gluzman, Marina Voikhanskaya, and Gary Low-Beer.

This request is unlikely to meet with official approval. Dr Gluzman is still in the Perm' labour camp, where he was sent for exposing the 'official' diagnosis of insanity in the case of General Grigorenko. Dr Voikhanskaya, who formerly worked in the hospital where Borisov is now confined, was obliged to emigrate (leaving her young son behind) after her repeated refusals to administer psychotropic drugs to dissidents. Dr Low-Beer, of Horton Hospital, Epsom, a prominent campaigner against the political misuse of psychiatry, told *Nature* that he is quite willing to comply with Mrs Borisov's wish that he should examine her husband, and that although it is virtually impossible that the Soviet authorities would allow such a commission to decide Borisov's fate, he himself would press for and demand the right of access to the 'patient'.

Unlike Borisov's arrest, the arrest and harassment of some 45 Jewish refuseniks in Moscow (including the physicists Mark Azbel and Aleksandr Lerner, the mathematician Viktor Brailovskii, and Academician Veniamin Levich, an electrochemist) is not directly associated with the release of Bukovskii. The group, members of the now-famous Sunday seminars for dismissed scientists, had been under pressure for some weeks following the announcement of their intention to hold a symposium on Jewish life and



Vladimir Bukovskii

culture in the Soviet Union. (A growth of Jewish consciousness is, not unsurprisingly, a common phenomenon among the refuseniks).

The group had been warned that if they persisted in going ahead with their plans, the authorities would take action. The threat was reinforced by a vicious attack in *Izvestiya* on Aleksandr Voronel, the founder of the group, who emigrated to Israel in December 1974; this called him a "former scientist without a future, indeed without a present", a "trader in human souls" and an "unmasked provocateur". The article recalled how, in summer 1974, Voronel's efforts to hold an international session of the seminar "failed completely"—as indeed it did, since would-be participants from abroad were refused visas and the "hosts" were arrested or subjected to severe harassment. A repetition of these tactics in the case of the Jewish symposium was therefore expected; it was hoped, however, that the authorities would intervene simply to break up the symposium, and that there would be no long-term arrests.

This, unfortunately, has not proved to be the case. Criminal proceedings have commenced against one of the would-be participants, the physicist Naum Salanskii from Vilnius, on the grounds that the paper he had prepared for the symposium slandered the Soviet Union by giving incorrect information about the treatment of the Jewish minority. In Leningrad, a Jewish mechanical engineer, Vladimir Shverdlin, was arrested on a charge of unlawful possession of weapons (apparently a few bullets left over from his membership, some years back, of a shooting club); although he has since been released, his family have been informed that the results of the investigation have been "passed on to the Procurator".

In spite of such warnings, the human rights campaign still continues. Following the arrest of Borisov, a group of eighteen Moscow dissidents has called

for a renewed campaign against the political abuse of psychiatry. In Kiev, a 'Helsinki monitoring group' has been set up, similar to that set up earlier

this year in Moscow; its members include Nina Strokata-Karavans'ka, a microbiologist, and the science-fiction writer Mykola Rudenko. □

USA

● President Ford's final budget, which is expected to be delivered to Congress on January 17 (just three days before his successor is inaugurated), will promise large increases in funds for research and development and propose that the green light be given to several important and eagerly-awaited scientific projects. Though it's possible that the whole budget document could be turfed out by the incoming Carter Administration, Mr Carter will have more pressing matters on his mind than tinkering with the nuts and bolts of the federal government's science programmes; it is therefore likely that some of Mr Ford's lame-duck proposals will survive more or less intact.

The rough outline of the proposed budget for research and development was sketched out last month by Guy Stever, the President's science adviser, who is also about to leave the government. Stever called a press conference after meeting to discuss the budget with Mr Ford, a number of prominent people from the scientific community and government officials from science agencies. He announced that Ford will propose that basic research be given a real increase, amounting to about 3% above the rate of inflation, in the fiscal year which begins on October 1, 1977, and that some of the increase would be spent on new projects in agricultural research, space science and earthquake prediction.

Following the pattern established in his two previous budgets, President Ford will propose particularly large increases in military science and technology and energy research and development. The proposed increase in military science alone will amount to about 15%, Stever said. Carter will take a close look at both those two areas, however, and Ford's proposals should therefore be taken with more than a pinch of salt.

As for space science, the proposed budget will contain some good news for astronomers and planetary scientists. Last year, in an effort to reduce public expenditure, the Ford Administration stripped about \$200 million from the budget of the National Aeronautics and Space Administration shortly before it was sent to Congress, and NASA was consequently forced to defer plans to build the Large Space Telescope (LST) and to start work on a spacecraft to orbit Jupiter.

Those funds will be reinstated in this year's budget, however, and if approved by the Congress and by the Carter Administration, the LST should be ready for launch in the early 1980s and the Jupiter mission will be launched in 1981. Both missions rank high on the list of priorities for space research drawn up by the National Academy of Sciences.



Another space project which Ford will approve is construction of a fourth satellite in the Earth Resources Technology Satellite series, called Landsat-D. The Landsat programme has consistently received strong support from Congress, and Ford's proposals will probably be accepted.

The lame-duck budget will also include a large helping of money for earthquake research, another area in which Congress has taken an interest. President Ford will propose that funds for earthquake monitoring and prediction be doubled in the next fiscal year, to reach about \$50 million. The money would be divided between the US Geological Survey, which operates a number of seismic stations and research establishments in California, and the National Science Foundation. No details are available of the projects which will benefit from this largesse, however.

Finally, in agricultural research, President Ford has decided to implement a proposal which has been advanced by three committees of the National Academy of Sciences and by two committees of the House of Representatives. He will propose the initiation of a new programme of competitive grants, funded through the Department of Agriculture, for basic

research related to agriculture. The new programme, which would receive about \$35 million next year under Ford's proposal, will complement rather than replace the Agriculture Department's traditional block grant system for agricultural research.

Usually, budget details are not released until the proposals are sent to Congress, but in this case, the tradition was broken presumably because the figures for research and development look good. Since Guy Stever and the Office of Science and Technology Policy can claim a good deal of the credit, they were understandably keen to get the word out before Stever departs.

● The National Academy of Sciences has finally added its voice to the chorus of criticism directed at the so-called 'hot particle' theory. Put forward in 1974 by Drs Thomas Cochran and Arthur Tamplin, staff scientists at the Natural Resources Defense Council, the theory is that tiny particles of inhaled plutonium may lodge in the lung and deliver a prolonged, intense dose of radioactivity to a small area of surrounding tissue, thereby posing a severe cancer hazard. NRDC argued that since present plutonium exposure standards are based on average doses of radioactivity to the entire lung, they underestimate the health hazards and should be tightened by a factor of about 115,000. Such strict standards would present a problem for reprocessing and plutonium fabrication plants.

In a report published last month, however, an Academy committee concludes that the evidence doesn't support the hot particle theory and that there is no need to lower plutonium exposure standards. The committee, which examined the theory under contract to the Environmental Protection Agency, looked at the results of tests on beagle dogs and concluded that the observed incidence of cancer doesn't fit in with the hot particle theory, and suggested that it "can adequately be accounted for by averaging the absorbed alpha radiation dose over the whole lung." The committee also argues that epidemiological evidence from experience with inhaled alpha-emitting particles suggests that the usual method of applying an average radiation dose to the whole lung to predict carcinogenic effects.

Colin Norman

FRANCE

Deceptively generous budget

The staff of La Recherche report from Paris on the funding for research in France in 1977

THE National Assembly and Senate of the French parliament have just approved the budget for scientific research for 1977—that is, they have approved the provisional calculations of expenditure for the 'enveloppe recherche' presented by the Minister for Industry and Research. But this budget package, since it does not take into account military research or telecommunications, is only a fabrication.

As early as the end of May last year,

the first draft of the 'enveloppe' had been strongly criticised by the consultative Committee on Scientific and Technical Research. M d'Ornano had then managed to get some adjustments to it through the Ministry of Finance. He finally obtained 10,935 million francs, an increase of 18% on last year's budget of 9,262 million francs. The major portion of this sum goes as usual to the Atomic Energy Commission (FF2,783 million), the National Centre for Space Research (FF1,014 million) and to information processing (FF725.6 million). The National Centre for Scientific Research receives FF2,555 million.

The budget is larger, however, only

because more jobs have been created. And it must be remembered that the package is already cut into by international commitments and by other expenditure unconnected with research. For the National Centre for Space Research, for example, FF586 million is reserved for multilateral programming, such as the programmes operated within the European Space Agency like Ariane, Marots and Spacelab. For information processing, on the other hand, FF450 million is allocated to CII-Honeywell-Bull.

The government has decided to create 950 new posts, of which 437 would be for research, and the rest technical or administrative. Furthermore, 900 people, 95 of them research workers, will benefit by the integration of 'hors statut' personnel. (Staff who

ISRAEL

● Israel's universities have spawned a new political party which threatens the local establishment more seriously than it has been threatened in many years. If present trends continue, the recently formed Democratic Movement of the Hebrew University archaeologist Yigael Yadin, and other reformist groups close to it, may well emerge as the strongest bloc in Israel's Knesset after the 1977 elections, perhaps even making it possible for Yadin to become the next Prime Minister. The elections are being held sooner than expected with the early end of Mr Rabin's government.

Yadin offers very little that is new in the policy sphere. Like large sections of the hitherto dominant Labour Party, he favours a compromise settlement with the Arabs, electoral reform and stringent measures to stabilise the economy. But he has the advantage of being a political outsider, untainted by the corruption and dissension that has beset Israeli public life in recent years.

This is not to say that Yadin has been an academic recluse in the quarter century since his retirement as Israel's second chief of Staff. On the contrary, his highly successful digs at Hazor in the Galilee and at Massada on the Dead Sea were well publicised, as was his service on such non-partisan bodies as the Agranat Commission (which investigated aspects of the Yom Kippur War). This makes him particularly attractive to the many Israelis who ardently desire "to throw the rascals out", but want to be sure that "the rascals" are replaced by men of experience and competence.

Yadin is by no means the only Israeli don to have played a part in public life: Israeli's first President, Dr Chaim Weizmann, was a distinguished chemist, and her current President, Professor Ephraim Katzir, headed the Weizmann Institute's Biophysics Department for many years. But the Presidency is largely a ceremonial post, and this is the only time that a savant has tried to exchange his academic robes for the powerful mantle of the Prime Minister.

● A central plank in Yadin's platform is his insistence that Israel remain a predominantly Jewish state, meaning that many of the Arab-populated areas now under Israeli control would, if he had his way, be relinquished within the framework of a peace settlement. This being the case, Yadin favours concentrating long-range development efforts on those parts of the country where the Arab population is sparse, with particular emphasis on the virtually empty Negev desert between Beersheba and Eilat.

Thanks to the discovery of a vast underground lake running from the Dead Sea to the middle of the Sinai Peninsula, it now appears that the Negev can be developed more rapidly and extensively than hitherto appeared possible. The Mekorot Water Company has carried out drillings in the wake of preliminary studies by scientists from the Ben-Gurion University of the Negev and the Weizmann Institute. Company officials speak of at least 200,000 million cubic metres of water at depths of between 800 and 1,000 metres. Initial tests indicate that the water has from 500 to 2,000 p.p.m. of minerals, which means that some of

it can be utilised immediately, while other water will have to be processed through reverse osmosis or electro-dialysis installations.

Water from the Negev 'lake' must be exploited carefully, as it is not likely to be replaced for quite some time. Most of it, according to carbon-14 dating tests, stems from the precipitation that occurred 20,000 years ago during the last Ice Age, when rainfall in the Negev averaged 1,000–3,000 mm a year, as compared to some 100 mm today.

● Rainfall now, it turns out, is heaviest where it is least needed, in Israel's urban areas. A recently completed study of climatic conditions in the Tel Aviv area over the last 30 years carried out by Dr Yair Goldreich of Bar-Ilan University and Alexander Mannes of Israel's Meteorological Service showed that rainfall had increased by 5–17% as compared to the rest of the countryside.

This study indicated that heat generated by greater radiation from concrete, asphalt and other built-up surfaces weakened the frequent temperature inversions of the air above the city, creating unstable conditions conducive to rain. At the same time, the greater currents of air caused by this heat and also by tall buildings contributed further to increased precipitation.

Another conclusion of the study was that growing air pollution in the Tel Aviv area, where population has tripled in the past 30 years, had caused a drop of 7% per decade in the amount of solar radiation reaching the ground.

Nechemia Meyers

are 'hors statut' are employed on contract for a limited period, whereas usually, in 'la recherche publique', researchers have the status of a civil servant, and are therefore guaranteed tenure).

There is also to be a large increase in the funds allocated to university research, due to go up from FF76 million in 1976 to FF120 million in 1977. This increase will rekindle the en-

thusiasm of the professors, who in return are urgently asked to put the affairs of the State Secretariat for Universities in order.

The research priorities of the Seventh Plan are, of course, the best safeguarded. Among these is research to reduce France's dependence on other countries for energy and basic materials. So FF195 million will be spent on providing new energy sources,

and FF45 million on uranium prospecting. In addition, special efforts are to be made to improve food production, scientific and medical instrument manufacture, prevention of pollution, and cooperation with underdeveloped countries.

It is indeed a budget which is misleading in its generosity, but which follows well-marked political intentions. □

SOUTH AFRICA

●South Africa is to embark on the construction of a 200 MeV open sector cyclotron early in 1977, thanks to the award of a government grant of R570,000 (£1=R1.45) for the next stage of the project. This is in addition to the more than R200,000 already spent on feasibility studies.

The facility, which will be built near Cape Town, has been 4 years in the planning and is expected to be operational in 1983. In due course the cyclotron and its staff will absorb the functions of the present Southern Universities Nuclear Institute at Faure, and progressively replace the CSIR's cyclotron, which is expected to have a lifetime of only a further 6 years. The new cyclotron will be used primarily for the production of radioisotopes for medical research, for radiotherapy, and for nuclear physics research.

Responsibility for the facility will be vested in an independent company, still to be set up, the funding for which is likely to come directly through the office of the Prime Minister's Scientific Adviser.

●The Prime Minister's Scientific Adviser, Dr S. Meiring Naudé, will retire at the end of the year. His successor is to be Dr A. P. Burger, a former director of the National Research Institute for Mathematical Sciences and currently a vice-president of the CSIR. Dr Naudé, a former president of the CSIR and one of South Africa's most respected scientific administrators, oversaw the introduction in 1973 of the procedure which is now followed for funding government-supported scientific research in South Africa. All requests for research grants are now channelled through the office of the Scientific Adviser, who alone pleads the case for state support for science with Treasury. Major research organisations in the public sector submit estimates for the coming year for both ongoing research and new projects.

Recent practice has been to award an across-the-board increase of at least 6% in grant aid to all appli-

cants mostly for ongoing research. As a means of exercising control on the direction of the national research effort, however, approximately 15% of the funds available have been assigned at the discretion of the Scientific Adviser to support projects recognised as being in the national interest. By this means, agricultural



research, which a few years ago was automatically given more research money than it had the resources to use effectively, now receives about 32% of the total compared with nearly half four years ago. In contrast, the budget of the National Institute for Metallurgy has been growing at about 25% annually, and that of the Human Sciences Research Council by at least 15%. Overall the national R&D budget has been growing by about 11% annually.

To help him in this sensitive task, Dr Naudé made use of an independent committee to set research priorities, consisting principally of eight senior scientists. Some were retired and others were attached to industry; none had a direct interest in the research organisations seeking support.

●Until it was closed down earlier this year, the deep space tracking station operated by the CSIR at Hartebeesthoek, 40 miles from Pretoria, had earned a reputation for being one of the most reliable of NASA's worldwide network of satellite tracking

facilities. Far from going out of business, the team of skilled engineers and excellent technical facilities built up during that period will now provide the basis for converting Hartebeesthoek into a monitoring station for remote sensing satellites.

The deputy president of the CSIR, Dr F. J. Hewitt, recently announced that plans are in hand to establish a line scan image processing facility service in conjunction with, initially, the Meteosat and Landsat satellites. When it becomes operational, Meteosat will play a crucial role in monitoring weather conditions over Southern Africa and the adjacent oceans. As for Landsat imagery, the new facility with its sophisticated electronic processing equipment will make it possible to collect remotely sensed data of much higher quality than is presently available from NASA in the form of transparencies of images recorded in different spectral bands.

The CSIR has become concerned that, in Southern Africa, too little advantage has so far been taken of what remote sensing satellites have to offer and, recognising that they are here to stay and bound to develop, is now to make a major effort to provide whatever facilities of hardware and software are needed to keep abreast of satellite technology. The CSIR will accept responsibility for the development of the more fundamental image processing methods, as well as for the provision of the usual infrastructural services at the tracking station. It is expected, however, that users will in time develop procedures for their own particular problems. Encouragement will also be given to sharing the facility with neighbouring territories, four of whom are already actively involved or interested in remote sensing.

Hewitt hopes that the station would be operational by August of next year, and that the sophisticated data processing and information extraction service will be available early in 1978.

Graham Baker

IN BRIEF

JET in difficulty

The hoped-for meeting of the EEC's Council of Research Ministers, provisionally scheduled for the week before Christmas, did not take place once it became clear that the Nine would again fail to agree on a site for the Joint European Torus (JET) project. The main obstacle seemed to come from France, which apparently intended to veto any proposed site other than Cadarache and suggested only CERN as an alternative. No date has been set for a future meeting. The view of the outgoing Research Commissioner Guido Brunner, that JET is "on its deathbed", is not accepted in Britain, where it is seen as an attempt to encourage an early decision.

Reprocessing developments

West Germany's recent indication that she will follow France and stop exports of nuclear reprocessing technology will not apparently cover her controversial deal with Brazil. France's own policy leaves unaffected her proposed export of a reprocessing plant to Pakistan, which is resisting pressure to cancel the deal.

Canada has meanwhile ended nuclear cooperation with Pakistan, as she did with India earlier last year, as part of a new exports policy just announced. In Britain the UK government has decided to subject plans for an oxide fuel reprocessing plant separately to a full public inquiry, but plans to expand facilities for Magnox fuel should re-

ceive an early approval. Elsewhere, Argentina has claimed she now has the wherewithal to produce nuclear weapons.

Research priorities decided

Specific areas of research in the field of gastroenterology are to be given priority by the UK Medical Research Council and the Department of Health and Social Security. Problems earmarked for the next 5 years include peptic ulcers, inflammatory bowel disease, infective hepatitis, cancer of the intestinal tract and gallstones; priority is also to be given to the study of gastrointestinal hormones, nervous control of the gut and the fundamental mechanisms of both intestinal absorption and pancreatic secretion.

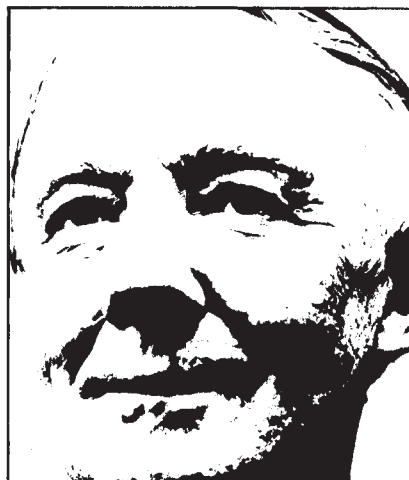
THE discovery and development of North Sea oil is already having a profound economic and social effect in those parts of Scotland where it is beginning to be brought ashore. Money is pouring in unprecedented amounts into areas which have always been poor. The extraction of oil is expected to reach its peak in the 1980s, and the reserves may be exhausted before the end of the century. While I tend to share the pessimism of the recent Nobel Laureate Milton Friedman regarding the probable effects of the oil on Britain's flagging economy, I am most concerned about the ecological effects of the industrial developments, and the possibility of pollution by oil spills, breaks in the pipelines and even by continuous low level seepages on the wildlife of many parts of the Scottish coast.

One area most likely to be affected is the Shetland Islands, the most northerly group of the British Isles, where gales and high seas make the work particularly hazardous. This hitherto undeveloped region, with a population of only 19,000, will have a terminal which will deal with as much as 150 million tonnes of crude oil in a year, more than the present total imports of mainland Britain. The likely results are summarised in a small book, *The Shetland Way of Oil* (Thuleprint, Sandwick, Shetland; 1976), produced by a group of mainly local writers. They describe without undue sentiment the way in which a life-style still cherished by at least the older Shetlanders is doomed, and suggest how permanent damage to the islands could be minimised. They are particularly aware of the limited volume of the oil reserves, and the need to exploit them rapidly to repay the enormous capital invested. They wish to prevent a temporary boom

declining rapidly and leaving ghost towns and derelict installations in a devastated environment.

The oil companies appear to be acting responsibly, and to be co-operating in trying to alleviate any

Shetland's oil



KENNETH MELLANBY

unavoidable damage. The cynical will no doubt suggest that this is in order to placate local interests which could delay development, but nevertheless the results are promising. The UK parliament has passed the Zetland County Council Act (1974) which gives this small local government authority financial powers rather greater than those proposed for the Scottish Assembly, and they should eventually have a very large sum of money, perhaps more than a hundred million pounds, to restore damage and provide industries and amenities when the oil is exhausted. It is thus likely that only the minimum damage will be done to the landscape, to buildings and to antiquities, and that means

will be found to encourage other industries to absorb those unemployed when the oil boom ends.

It is difficult to be so optimistic about wildlife, for in an operation of this size some serious pollution is almost inevitable. The good thing about the oil rush is that it has made conservationists aware of the richness of the flora and fauna of Shetland and of Scotland generally, and of their ignorance of what is actually there. It has stimulated ecological surveys by and on behalf of the Nature Conservancy Council, which show us what might be lost. Plans are being made to monitor any changes which occur during the exploitation of the oil, so that damage may be quickly assessed, even if it cannot always be prevented. Attempts are being made to find ways of minimising any damage if leaks or spills occur. Some surveys were not completed soon enough to prevent shore works being carried out in valuable wildlife areas, but some attempt to avoid these has been made. The problem of the ultimate rehabilitation of damaged habitats will also be studied.

Fishing, farming and knitting are traditional industries of Shetland. Already oil has contributed to the reduction in fish caught, though over-fishing and international politics have also had their effect. High wages at the oil terminal take labour from the farms and the knitting factory. It is hoped that, when the oil is exhausted, these and other industries will be restored and developed. If there is a world energy crisis there should be an assured market for Shetland knitwear, including the Fair Isle pullovers so popular in the 1920s with the then Prince of Wales, to keep us warm when we can no longer afford to over-heat our buildings.

correspondence

The scientific civil service

SIR,—Your leading article of November 18, "Don't Move", is itself guilty of throwing "the same old accusations" at the scientific civil service without any supporting evidence. The IPCS would certainly agree that the scientific civil service still lacks the right openings for mature scientists whether from within or without. It cannot, however, accept that the scientific civil servant is considerably better paid than his counterpart elsewhere; nor do scientists in the civil service feel particularly secure in their jobs at a time when cuts in public expenditure are raising real threats of redundancy.

It is of course true that industrialists frequently complain that they find it difficult to recruit in competition with the service; they are less willing to make public the details of the terms they themselves offer to scientists so that comparisons can be fairly made. Meanwhile the trend away from science continues in both the schools and the universities. The recent report from the Select Committee on Science and Technology, "University-Industry Relations", underlines the importance of reversing that trend. It will not be done by continued sniping at particular employment sectors but by improving the status of engineers and scientists in the community at large and, as the Select Committee recognises, by giving them "the opportunity of moving into the top echelons of management with as much ease as their counterparts in Germany, France or the USA and as their counterparts in Great Britain who have chosen to study law or accountancy".

Greater movement of scientists between different sectors of employment, though desirable, will not of itself solve the underlying problem of the un-

attractiveness of science as a career—whether in industry or the civil service.

MARGARET PLATT

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International contacts

SIR,—I would like to complete Professor Burhop's article ("A case of suitable treatment?", November 11) by one relevant remark. At the same time as London created obstacles in issuing British entry visas for some delegates of the World Federation of Scientific Workers' General Assembly, the former vice-president of the Federation and a long term head of its Prague Regional Secretariat, Academician Ivan Málek, was visited by two gentlemen from the Prague police, who confiscated his foreign passport, valid only for East European countries—perhaps from fears that he might try to participate in the Pugwash meeting in August in East Germany, or the London Assembly.

I fully understand Professor Burhop's indignation about the bureaucratic obstacles to realising international scientific contacts. What I don't understand is why he does not publicly condemn equally strongly the incomparably heavier fact that thousands and thousands of scientists from the USSR and Eastern European countries are extremely restricted in travelling abroad, that every single journey must be approved and depends fully on the goodwill of the authorities and political views of each applicant?

Professor Málek, a scientist with an international reputation and a member of many international organisations, committees and boards, has not been allowed to leave Czechoslovakia for

over 6 years (although he is constantly being invited abroad), and only because of his disapproval of occupation of Czechoslovakia in 1968 by Soviet and "allied" armies.

FRANTIŠEK JANOUGH

*Stockholm,
Sweden*

USSR river diversions

SIR,—There is a disturbing aspect of the Soviet plan to divert the rivers Irtysh and Ob southward (December 2, page 390) which your correspondent does not mention. Calculations reported by Aagaard and Coachman in *Eos* (56, 485) suggest that by removing or reducing the low salinity layer at the surface of part of the Arctic Ocean such a scheme could allow deeper convection of the surface waters, encouraging ice to melt with perhaps a positive feedback as the albedo of the region is thereby reduced. Likely results, they suggest, "would be (1) prolonged ice-free conditions because of deep-reaching free convection . . . (2) the release of large amounts of heat from warm Atlantic water during the cold months, and (3) the elevation of quite saline water into close proximity with the sea surface".

It is not clear, of course, whether such effects should be regarded as desirable or undesirable; in the present state of the art regarding our understanding of climatic changes we can only say that repercussions of one kind or another on the general atmospheric circulation would be inevitable. Given the uncertainties involved, however, it seems most unwise to carry out the experiment.

JOHN GRIBBIN

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Competition 11

Readers are invited to submit descriptions, in epigrammatic reportorial form, of the demeanour, condition or action of any of the famous scientists (past or present) participating in an imaginary general symposium. Submissions should offer some humorous association with the

scientists' work, for example, "Faraday was incapacitated", "Boyle and Charles felt the meeting was 'a perfect gas'". Closing date for entries: February 16. (Competition inspired by Charles S. Cope of Parkersburg, West Virginia.)

Competition 10. "Prehistoric camels often sit down carefully. Perhaps their joints creak. Early oiling might provide permanent relief". These memorable phrases earn Dr P. Little, of Wantage, UK, £10 as the best

mnemonic of a rather disappointing bunch. They remind us that the geological epochs are Precambrian, Cambrian, Ordovician, Silurian, Devonian, Carboniferous, Permian, Triassic, Jurassic, Cretaceous, Eocene, Oligocene, Miocene, Pliocene, Pleistocene and Recent (with apologies to the Paleocene).

As we said to our accounts department, "Prepare cheque of suitable denomination. Competitor pleases the judges, conquering easily over many people's poorer reminders".

news and views

Interpreting Stonehenge

from R. J. C. Atkinson

THE formulation of astronomical interpretations of Stonehenge is becoming a minor industry with its own characteristics. These are well illustrated by a recent and a current contribution to *Nature*.

R. F. Brinckerhoff has suggested (*Nature*, **263**, 465; 1976) that selected depressions on the tops of some of the lintels were made to hold markers used by an observer, himself standing on the lintels on the opposite side of the circle, to identify significant risings of the moon. This is clearly possible under certain circumstances; but is it likely?

I think not, if only because the tops of the lintels are now so weathered as to make it impossible to distinguish artificial holes from those of natural origin. On other lintels, notably stone 152, there are many weathered holes far deeper, and no less regular in outline, than Brinckerhoff's examples, for which no astronomical explanation is possible. Furthermore, the author admits that the relevant observations could have been made only after the fall of stone 55. The date of this event is unknown; but we do know from Gowland's excavations of 1901 (*Archaeologia*, **LVIII**, 37–118; 1902) that the base of this stone was not deliberately undermined. The differential weathering of the upper and lower surfaces of the lintel which it formerly supported (stone 156) suggests that stone 55 may not have fallen until well after the end of the Early Bronze Age. There is no archaeological evidence for the continuing use of Stonehenge thereafter.

A. D. Beach (this issue of *Nature*, page 17) makes the new, ingenious, but to me equally improbable suggestion that the Aubrey Holes were used as counters for predicting tidal amplitudes by way of previous knowledge of the period of rotation of the lunar apsides. This is a more 'difficult' explanation than Hoyle's (which Beach apparently rejects) because it involves an additional generalisation from natural phenomena unobservable at Stonehenge itself.

From our present knowledge of celestial mechanics it is all too easy to impute to our prehistoric ancestors an understanding of nature which may well be ours alone. Both these papers, by concentrating exclusively on the astronomical interpretation of aspects of a monument which was surely more than an observatory (if it was ever that), lead to invalid inferences and to the posing of non-problems.

Brinckerhoff says, for instance, "No one has ever explained convincingly why it [the linteltop walk-way, so-called] had to be 16 feet high." Why indeed, except that the builders so decided within the limits of the building material available? Has anyone, for that matter, explained convincingly the height of the spire of Salisbury Cathedral? That the lintel-tops could have been used as an elevated walk-way does not necessarily imply that they were.

Beach, in advancing a purely 'functional' purpose for the Aubrey Holes, dismisses the 'religious' explanation of

Stonehenge I as "surely the lowest common denominator of imprecision and evasion". One wonders how he accounts for the use of Stonehenge I as a cemetery, and for the occurrence of similar cremated burials in similar rings of pits elsewhere in Neolithic Britain, for which no 'functional' purpose has ever been suggested. In fact, of course, one need look no further than many Christian churches, orientated on the sunrise either at the equinoxes or the patronal saint's day, to see that a religious purpose need not be incompatible with some elements of astronomy; nor does the incorporation of sun-dials and clocks in churches imply that they were built primarily as time-pieces.

Both these papers seem to me to show an almost obsessive desire to interpret Stonehenge exclusively in 'practical' or 'scientific' terms, and to ignore the probability that it served simultaneously a number of purposes which only we would regard as separable or conflicting, and many of which, could we know them, we should find it virtually impossible to understand. The distinctions which we make between the rational and the irrational, the practical and the useless, or between religion and science, are all part of a universe of discourse which is quite inapplicable in a prehistoric context. To impose that universe of discourse on our remote ancestors is to abolish history, and to people the prehistoric past with ourselves in fancy dress. □

Metal bites metal

from Robert W. Cahn

THE recent Report of the Court of Inquiry (HMSO, London, 1975) on the disaster at Flixborough, where a caprolactam manufacturing plant exploded with grave loss of life, cited surprising evidence concerning weakening of hot stainless steel by traces of metallic zinc on the steel surface. This had not been recognised before the disaster inquiry and the special research commissioned

to help in that inquiry. It appears that minute quantities of zinc, which can reach the steel either as liquid or as vapour, lead to fracture within seconds at modest stresses ($5\text{--}6\text{ kg mm}^{-2}$) in the temperature range $800\text{--}900^\circ\text{C}$. Without the zinc, a stress well over 10 kg mm^{-2} would be needed in this temperature range to achieve similarly rapid fracture. This is an instance of stress-

corrosion, a phenomenon which involves the mutual abatement of stress and corrosion: the corrosive agent accelerates the action of the stress and the stress enhances the corrosive attack. The importance of zinc-embrittlement in the sequence of events that led to the explosion was the central and highly disputatious issue at the Court of Inquiry; mutterings of dis-

agreement as to the official findings may still be heard today.

Stress-corrosion by liquid metals takes place far more commonly than was recognised two decades ago: the subject has been comprehensively reviewed by M. H. Kamdar (*Prog. Mater. Sci.*, **15**, 289–374; 1973). Generally, such attack is not pronounced at or near ambient temperature, and it is thus a form of brittle fracture, enhanced by adsorption of the contaminant at the root of surface cracks. Some trace metals embrittle a variety of solid metals: thus mercury embrittles aluminium, zinc, cadmium, iron, silver, titanium and others, gallium attacks most of these metals (most spectacularly, aluminium), whereas bismuth only embrittles copper, and cadmium only iron and titanium. Mercury can claim championship status as a general trace nuisance in biology and engineering alike!

A paper by W. T. Grubb in this issue of *Nature* (Grubb, page 36) adds a further example to the growing number of instances of liquid metal embrittlement. It resembles the stainless steel/zinc syndrome in that the embrittlement is specific to high-temperature stressing and does not arise at room temperature, unlike the general run of such cases. Grubb has discovered that zircaloy-2 (a zirconium-base alloy containing 1.5 at.% tin and small amounts of iron, chromium, nickel and oxygen, which has long been used to enclose fuel elements in water-moderated nuclear reactors) is embrittled by traces of metallic cadmium—solid, liquid, or dissolved in another liquid metal—in the narrow temperature range 300–340 °C. The fracture is purely brittle in character under these circumstances and takes place by cleavage on the (hexagonal) basal plane. The report is clearly preliminary and there is as yet no information about the time during which stress must be applied to generate fracture: it is a

frequent characteristic of stress-corrosion that fracture is delayed.

This finding follows the discovery, ten years ago, that iodine can cause stress-corrosion failure in zircalloys (there are several variants of the alloy) in the range 250–500 °C. J. C. Wood (*J. Nucl. Mater.*, **45**, 105; 1972) studied the matter in detail for zircaloy held at 300 °C, which is a typical reactor-operating temperature for water reactors. For adequately large stresses and surface concentrations of iodine, fracture ensued, but previous anneals to remove residual internal stresses greatly reduced its incidence. Iodine-embrittlement is of peculiar concern because iodine, a volatile element, is a major fission product and is thus created inside the fuel element during service. Other fission products, however, would be expected to 'getter', that is, neutralise any free iodine; different metals can be regarded as being in competition for the attentions of free iodine. There was, in 1972, no clear evidence that any cracks in fuel element containers had been caused by this mechanism, and I have seen no later papers to suggest that any such events had been established. Wood's paper pointed the way to a number of precautionary measures.

Cadmium-embrittlement of zircaloy might conceivably be of consequence in a nuclear reactor because cadmium is one possible constituent of neutron-absorbing control rods, and Grubb's discovery may serve to discourage the use of this particular neutron absorber.

More generally, it is now clear that liquid metal embrittlement (if we may regard iodine as an honorary metal for present purposes) falls into two clearly distinct categories: hot or creep embrittlement, and cold embrittlement. It will be the next task of background research in this field to establish the characteristics of aggressor/victim combinations that predispose a system to one of these two modes of attack. □

success of cultural traditions, which goes against the intuition of an evolutionary biologist well schooled in the view that differential survival of groups is not important in genetic evolution.

Recently W. H. Durham (*Q. Rev. Biol.*, **51**, 385; 1976) has discussed the cultural evolution of traditions of inter-group warfare in man, attempting to analyse the problem in terms of costs and benefits to individuals rather than groups. In animals, aggression is almost invariably associated with competition for resources such as food and mates, and whether or not fighting evolves as a means of competition depends on the costs and benefits to individuals (see below). Resources which are too widely scattered to be economically defensible are not usually exploited by aggressive competition. Durham suggests that this may be why Eskimoes living off migrating caribou and salmon have little tradition of intergroup hostilities, while those in South West Alaska living off more reliable sources of game do have traditions of intergroup warfare. In considering group conflicts, Durham argues that the payoffs to an individual will vary with the group size. A larger group will have more chance of winning, but there are more individuals to share out whatever resource is captured through the victory. Although the details may be complex, these trade-offs will lead to an intermediate optimum group size from the point of view of an individual in the group.

Durham discusses the example of the Mundurucu of Brazil in detail. These people traditionally lived in villages of about 200 individuals and engaged in intergroup warfare, collecting heads of rival villagers as trophies. The classical anthropological interpretation of this practice is that head hunting was a

Economics of aggression

from John Krebs

THE idea that cultural and genetic evolution are analogous is not new: both processes consist of the survival of a few out of many possible competing entities—ideas in the former case, genes in the latter. Some anthropologists and biologists have used this obvious parallel to develop the argument that human cultural traditions, like genes, have survived because they are adaptive (Harris, *Culture, People, Nature; An Introduction to General Anthropology*, Crowell, New York,

1971; Blurton-Jones, in *Growing Points in Ethology*, Cambridge University Press, 1976). The difficult question is to decide what is meant by adaptive. In the case of genes the answer is easy: successful genes survive because their individual owners are good at surviving and reproducing (Dawkins, *The Selfish Gene*, Oxford University Press, 1976), but do cultural traditions survive for the same reason? Most discussions by ecological anthropologists have emphasised group survival as a criterion of

Correction

In the report "Control systems in higher plants" (*News and Views*, **264**, 214; 1976) work of Daphne J. Osborne and coworkers was incorrectly described. The last sentence of paragraph 1, page 215 should read: "The rate of germination of normally slow (low vigour) embryos is however increased by a preliminary hydration, because during this period part of the early stage of germination is achieved. The biochemical changes are maintained when the embryo is dried again, so that when next supplied with water, such embryos complete germination more quickly, or are said to be 'enhanced'. In other words, if this treatment is given to whole seeds, a higher percentage will then emerge above the soil."

PLANETARY tides on the Sun influence solar activity, which in turn causes an increase in the solar cosmic ray flux reaching the Earth's upper atmosphere. This triggers unusual movements of large air masses, thus effecting the Earth's rate of rotation and subsequently triggering earthquakes. This, in a nutshell, is known as the 'Jupiter effect' mainly because Jupiter exerts the largest tidal influence on the Sun (this influence is proportional to Md^{-3} where M is the planetary mass and d its distance from the Sun, so that Mercury, Venus, Earth, Mars, Jupiter, Saturn, Uranus, Neptune and Pluto have relative tidal effects on the Sun in the ratios of 4.1 : 97.3 : 44.2 : 1.4 : 100 : 49 : 0.09 : 0.03 : 0.001 assuming the Jovian effect to be 100.) It was first proposed by K. D. Wood (*Nature*, **240**, 91; 1972) and popularised in *The Jupiter Effect* by John Gribbin and Stephen Plagemann (Macmillan London; Walker, New York; 1974) which generated considerable discussion and also a degree of criticism, counter argument and rejoinder (*Icarus*, **26**, 257, 268, 270; 1975).

Jean Meeus, the most severe critic, pointed out that according to this interpretation of the data, planetary alignments have an undetectable effect on sunspot activity. In his opinion the effect doesn't exist. But Gribbin and Plagemann's argument is that planetary alignments are important and none more so than the 1982 superconjunction when Jupiter, Saturn, Uranus and Neptune will be colinear. These superconjunctions occur about every 178.73 yr. In this

Planetary alignments don't cause earthquakes

from David W. Hughes

time Jupiter travels 15 times round its orbit plus an extra 24° , Saturn performs six revolutions plus 24° , Uranus two revolutions plus 46° and Neptune one revolution plus 30° . Meeus points out that a period of 178.73 yr should have no special significance as Saturn, Uranus and Neptune exert a rather minor tidal influence on the Sun in comparison with, say, Venus and Earth (see above). Tides raised on the Sun by Venus, Earth and Jupiter have a period of four months and this should be the dominant factor if any interrelation exists. The temporal similarity between the 11.1-yr cycle of solar activity and the 11.86-yr orbital period of Jupiter is thought to be entirely fortuitous. Also there are two tidal bulges produced on the Sun, one at the sub-planetary point and the other diametrically opposite, so the planets don't have to be all on the same side of the Sun as was asserted by Gribbin and Plagemann. The heliocentric opposition of January 16, 1901 should have been just as effective as the forthcoming superconjunction of 1982.

Despite these arguments Gribbin and Plagemann still "see no reason yet to discard the suggestion that planetary alignments do affect sun-

spots, let alone the more fundamental contention that solar activity affects the Earth's rotation and therefore earthquakes."

One way to solve this disagreement is to look at earthquake data to see whether the occurrence of earthquakes has a periodicity of about 179 yr. Gribbin and Plagemann peer into the future, predicting that 1982 is going to be a year of considerable activity and a possible date for the unwelcome movement of the San Andreas fault in California.

Also addressing this problem W.-H. Ip of the Department of Applied Physics and Information Science, University of California, San Diego, does without the crystal ball and takes a more orthodox scientific approach. In a recent issue of *Icarus* (**29**, 435; 1976) he describes how Yu Shen (in *Ke Yue Shi Yan* (*Scientific Experiments*), May 1975 page 22) has divided the period between 1000 AD and the present into quiet and disturbed periods of earthquake activity using ancient records of earthquakes in Northern China. Ip finds that since 780 BC only one of the 125 recorded earthquakes with intensities corresponding to 6 on the Richter scale coincided with a heliocentric planetary alignment, this one occurring in 1624 AD. Since 1000 AD none of the eleven earthquakes with intensities of 8 has coincided. These facts really do speak out against the Jupiter effect and Californians can, one hopes, rest more easily in their beds; San Andreas will undoubtedly move some time in the future but 1982 is no more likely to be the year than any other.

cathartic activity promoting within-group harmony although, as Durham points out, it seems a rather extravagant method of promoting peace within the village. Instead, he suggests that the tradition is directly related to competition for a scarce resource: game animals. The Mundurucu rely on game for high quality protein, and this is reflected in the importance of game in their religious rituals. Head hunting rituals also involved reference to game. A successful warrior was referred to as 'mother of the Peccary', referring to the fact that other village members were viewed as equivalent to game, and that obtaining a head trophy magically increased the fertility of game animals. This fits in well with Durham's interpretation that intergroup warfare is a form of individually selfish competition for game. More puzzling from the point of view of individual benefits, is the tradition that a successful warrior abstains from sexual intercourse for three rainy seasons. Not much good for individual fitness!

Durham argues that the very scarcity of protein which precipitated the warfare would make procreation at that moment an unprofitable business (he doesn't say whether the whole tribe abstains), and that the warrior's payoff comes in other ways, such as exemption from future raids.

Durham's approach to interpreting cultural traditions of warfare is entertaining and makes intuitive common sense to a biologist, but whether it will really provide any predictive insight into differences between cultures rather than merely explaining after the fact, remains to be seen.

Returning to the costs and benefits of aggressive competition for food in animals, Carpenter and MacMillen, (*Science*, **194**, 639; 1976) have recently reported a quantitative study of territoriality in the Hawaiian Honeycreeper *Vestiaria coccinea*. Individuals of this species sometimes defend a feeding territory on flowering trees of the genus *Metrosideros*. Carpenter and MacMillen argue that territorial aggression

will be economically advantageous as a means of securing food only if the gain in terms of increased nectar yield through excluding competitors exceeds the energy cost of territorial defence. Thus, for a given efficiency of excluding nectar thieves, territoriality will pay only if the average nectar productivity is above a certain level. (If productivity is low, no amount of exclusion of rivals will pay for itself.) At very high levels of productivity, there is so much nectar available that exclusion of rivals is not necessary, so honeycreepers should only defend food territories at intermediate levels of nectar production. By calculating the energy cost of maintenance for a territorial and non-territorial bird, as well as estimating the nectar production in each territory and the efficiency of exclusion of rivals, Carpenter and MacMillen could predict in a quantitative manner which birds should show territorial aggression, and which individuals should not. For nine out of ten birds (six territorial and four non-territorial) the behaviour was

as predicted by the model. These results show how a simple cost-benefit theory can predict (as opposed to Durham's *post hoc* explanation) the occurrence of territorial competition for food in honeycreepers, although the authors suggest that an important additional factor is that the territorial birds defend more food than they need in the short term, perhaps as an insurance against hard times. □

Man's impact on nature

from Egon T. Degens

A Dahlem Conference on Global Chemical Cycles and their Alterations by Man was held in West Berlin on November 15–19, 1976. It was organised by Dr Silke Bernhard from whom further details may be obtained at Dahlem Konferenzen, D-1000 Berlin 33.

WHEN man started to cut forest, burn steppe and plough land, the balanced system of air, water, life and soil was gradually disturbed. Through this type of land management, soil erosion rates quadrupled over the past 2,000 years and were borne with all their side effects by the environment, almost unnoticed by man. Industrial revolution and a growing population accelerated man's insult to nature to a point that the potential dangers are now inescapably apparent. Concerned people throughout the world are asking the now familiar questions: what is the present state of the global environment, where are we heading if increase in gross national product has higher priority than conservation, and what immediate steps are to be taken to improve the quality of the environment? At the most recent of the Dahlem Konferenzen, social and natural scientists addressed themselves again to some of these questions, though it was interesting to note that no member of the third world countries attended.

Some fourteen background papers (most of which, in my opinion, were rather catholic, indicating that we cannot yet give precise answers to global enquiries) elicited hundreds of written comments and questions. It was frustrating that only a few could be picked up in the general discussion.

Four groups addressed themselves respectively to the topics of models, modulations, and controls; fossil fuel burning and its effect on the biosphere and on sedimentary cycles; source functions: combustion products of fossil fuels, radionuclides and heat; and the resilience of the environment and of human society to changes in

energy use. But it turned out that there is no way to deal reasonably with such an involved bundle of items, at least not during a five day session. Each group independently selected a few critical areas for discussion and almost identical key issues came up in the four huddles—man's impact on the global carbon, nitrogen, oxygen, phosphorous and sulphur cycles, and the interaction of C–N–O–P–S compounds with nature through these cycles.

Questions of immense importance turned out to have tremendously large uncertainties. One example may be taken—the question of whether global biomass is increasing or decreasing? Geochemists say it must increase, because they have a hard time finding another potential sink for the 'missing CO₂' in the atmosphere. This is so because, of the 4.6×10^9 tons of combusted carbon emitted every year as CO₂ into the air, only about 50% remains there; the rest apparently goes elsewhere, and it's not into the ocean. In contrast, biologists say the global biomass may remain steady or even decrease, partly as a result of excessive cutting of wood. They even believe that deforestation and the accompanying oxidation of wood and humus is a significant contributor of CO₂ to the air, and values between 1×10^9 and 5×10^9 tons of C could be annually released to the air if one uses some of the published figures.

So it seems that the increase in atmospheric CO₂ is not solely due to the burning of fossil fuel but that deforestation is also an important pollutant. How can we reverse this trend? Should we plant a trillion trees, as somebody suggested? But to be realistic, there is no way out than to cut CO₂ emission to the atmosphere over the next 20 years or man will be warm but very, very sorry. Although the climate curve shows a cooling trend at present, only a minority of one-and-a-half was of the opinion that the impact of man-made CO₂ on climate is beneficial. As well as producing CO₂, fire and fossil fuel burning also release particles to the air. How do these affect global climate? Does stratospheric light-scattering by particles lower world temperatures or are the particles more efficient absorbers than scatterers and thus raise the global temperature? No opinion one way or the other was arrived at.

Equally uncertain were questions of the significance of man's perturbation of the ozone layer compared with natural perturbations. What are the quantitative effects of aerosols, N₂O, or CO? It turned out that the data base for ozone is not good. There are not only chemical but meteorological changes and not all participants shared the view that man causes the altera-

tions noticed in the ozone layer. Are we kidding ourselves, because we ask questions on these and related issues that we cannot answer? The whole reputation of science is at stake, because in the past so much has been made of spray cans, fuel combustion and other environmental hazards caused by man's activities. The scientist's role is to bring about a change in technological approaches, which may lighten the burden on nature and at the same time help to fulfill the social needs of all the people in the world, and not just those from the developed countries. To accomplish this the scientist needs to put forward an exceptionally credible case, because the biggest inhibition of public action is disagreement among scientists.

In conclusion one of the finest results of the Dahlem Konferenz was that all participants tried to agree on the range of uncertainties, rather than being disappointed at not coming to a scientific consensus. There is a long way to go before global models of chemical cycles are universally accepted. At present the question of man's impact on sources and sinks of the biogenic elements is still up in the air.

Why marsupials can't win

from Barry Cox

ONE of the most intriguing problems in vertebrate evolution is the reason for the superiority of eutherians ('placentals') when competing with marsupials. Because they are so alike in their levels of morphological adaptation, it has long seemed likely that the cause lay within the fundamentally different patterns of reproductive physiology of the two groups. In an extremely interesting paper, Lillegraven (*Evolution*, **29**, 707; 1976) has recently examined this possibility.

Lillegraven commences by reviewing a number of papers by Müller, whose contributions have been largely ignored (unread?) by English-speaking workers. Marsupials are born nearly helpless, at a very early stage of development, since they have a very short period of gestation. Müller has shown that their pouch young show several features adapted to life in the pouch, such as seals to close the lips around the nipple, to protect the eyes and to plug the external auditory meatus. All these adaptations occur fleetingly in the development of eutherians, strongly suggesting that they, too, originally had a developmental history like that of living marsupials.

Although Müller herself regards the marsupials and eutherians as having

developed their reproductive strategies independently from an oviparous common ancestor, Lillegraven rejects her reasons for this conclusion. His own earlier work (*Palaeont. Contribs Univ. Kansas*, **50**, 1: 1969) has shown that a comparison between the details of the structure and development of the membranes and early embryos of a didelphid marsupial and a eutherian show no features that exclude the marsupial system as phylogenetically ancestral to the eutherian system. Even the marsupial pouch is absent in such forms as the didelphid *Marsoma*, whose young swing freely from the nipples, and Lillegraven suggests that such a developmental history was present in the common ancestor of marsupials and eutherians. Such a relationship is also supported by modern palaeontological work that Müller had not considered.

Most marsupials have a gestation period of only 11–15 d. Though in some this is extended up to 38 d, this extension is not accompanied by a corresponding advance in the stage of anatomical development that has been reached at birth; instead it is accompanied by a slow-down or standstill in development. It seems as though there is a natural limitation to the amount of growth and development that can take place within the marsupial uterus, so that beyond this point the embryo must be transferred to the extrauterine nipple.

Lillegraven suggests that this limitation is imposed by the natural incompatibility between the 'foreign' foetal tissues and the tissues of the mother. Intimate relationships between these two tissues would trigger the highly efficient immune response, leading to immunological rejection by the maternal haematopoietic system. Though a placenta does develop in some marsupials, this problem restricts the duration and intimacy of relationships between foetal and maternal tissues. So the marsupial embryo can develop within the uterus only until its own food reserves in the yolk and abdomen are exhausted, and then must be born before the mother can mount a fully-fledged immunological attack.

The ability of eutherians to avoid this immunological crisis is due to the evolution of the trophoblast, which provides the major barrier between the foetal antigens and the maternal antibodies. At least one trophoblast layer always lies between foetal and maternal tissues within the placenta. The mechanism of inhibition of the maternal immune response is still uncertain, and seems to include physical separation by non-cellular trophoblasts, the development of tolerance within the mother due to transfer of materials from the

placenta, and the active involvement of the trophoblast in opposing rejection of the foetus. These adaptations made it possible for the placenta to be used for respiratory and excretory exchange and for nutrition of a yolk-less embryo over a prolonged period of time. The trophoblast also transmits maternal immunoglobulins to the foetus, and is responsible for synthesis of hormones allowing prolongation of pregnancy. As a result of all these adaptations, the eutherian young can be born at an advanced stage of development, and already possessing immunity to some infections.

As Lillegraven points out, the marsupial type of development also limits that group to producing a neonate that can independently reach the nipple and survive there breathing air. This prohibits the evolution of fully aquatic marsupials, or of marsupials in which the forefeet are specialised into flippers, non-opposable fingers or single-toed hoofs. (This perhaps explains why the Australian answer to the eutherian ungulates is the kangaroo.)

At least, with living marsupials to examine and compare with their eutherian relatives, it is possible to identify such a biochemical reason for their competitive inferiority. If marsupials were extinct, palaeontologists today would be faced with an insoluble problem! □

Ligand binding properties of the haem group

from A. J. Thomson

DEOXYMYOGLOBIN and deoxyhaemoglobin contain the paramagnetic form of the haem group as a result of the presence of the high spin ferrous ion. It is now accepted that in this spin and oxidation state the iron sits out of the plane of the haem since it is too large to fit into the hole in the ring. On binding oxygen these proteins become diamagnetic and the iron atom drops back in the centre of the haem ring. It has been proposed, in the case of haemoglobin, that, on binding oxygen, this movement of the iron atom triggers a change in the quaternary structure of the protein which leads, in turn, to an increase in the oxygen affinity of the remaining haem residues in the protein. This mechanism is thought to be the molecular basis for the Bohr effect (Perutz, *Nature*, **228**, 726; 1970).

The nature of the oxygen-iron binding has been controversial in two respects, however. First, the stereochemistry of the iron-oxygen unit has, until recently, been much debated in

the absence of good structural data. Second, the formal oxidation state of the iron atom has been in dispute.

From X-ray studies of the synthetic model compounds 'picket-fence' porphyrins ($\alpha, \alpha, \alpha, \alpha$ -tetrapivalaminophenylporphyrin, TpivPP) which bind oxygen reversibly, it has been determined that the dioxygen binds iron in a bent end-on fashion with a Fe-O-O angle of 130° (Collman *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1326; 1974). But the formal oxidation state of the iron has proved more difficult to resolve.

The problem was to distinguish between two possible states. Pauling (*Nature*, **203**, 182; 1964) has described the system as low spin ferrous iron bound to dioxygen which has become spin paired. This presumably implies little transfer of electronic charge from the iron to the dioxygen ligand. The second view first put forward by J. J. Weiss (*Nature*, **202**, 83; 1964) suggested that one electron is transferred to give a species $\text{Fe}^{\text{III}}\text{-O}_2^-$, the low spin ferric ion and a superoxide anion, with their electron spins anti-ferromagnetically coupled to give a diamagnetic resultant.

To probe the nature of the bound oxygen ligand and to distinguish between a superoxide ion and a neutral dioxygen the infrared stretching frequencies have been measured. This frequency drops steadily as negative charge is added (for example O_2 , 1,556 cm^{-1} ; O_2^- , 1,150–1,100 cm^{-1} ; O_2^{2-} , 740–850 cm^{-1}). Caughey and his coworkers (*Biochem. biophys. Res. Commun.*, **55**, 91; 1973; *Biochem. biophys. Res. Commun.*, **58**, 166; 1974) in pioneering experiments succeeded in measuring the dioxygen stretching frequencies in both oxymyoglobin and haemoglobin, obtaining values of 1,103 and 1,107 cm^{-1} respectively. On substitution with the isotopically-labelled species $^{18}\text{O}_2$ shifts to 1,065 cm^{-1} were seen in both cases, adequately confirming the assignment of the bands. Although these values clearly lie in the superoxide range Caughey and his collaborators do not favour a formulation as ferric superoxide, rather they prefer to talk of strong covalent bonding between the iron atom and dioxygen. Initial attempts to measure the oxygen stretching frequency in the model Fe-TpivPP compounds produced a value of 1,385 cm^{-1} (Collman *et al.*, *J. Am. chem. Soc.*, **96**, 6522; 1974). It is now known, however, that this result was an artefact caused by an unknown impurity in a KBr disk. Recent measurements place the value at 1,163 cm^{-1} in a $\text{Fe}(\text{TpivPP})(\text{NTrIm})\text{O}_2$ model and 1,159 cm^{-1} in $\text{Fe}(\text{TpivPP})(\text{NMeIm})\text{O}_2$, where NTrIm is 1-tritylimidazole and NMeIm is 1-methylimidazole. Isotope shifts on substitution with $^{18}\text{O}_2$ are used to confirm assignments (Collman *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 3333;

1976). This revision brings the model compound values close to the haemoprotein values, all now lying within the superoxide range.

A comparison can usefully be made with a range of cobalt-porphyrin derivatives which also bind oxygen reversibly. Caughey and Maxwell (*Biochem. biophys. Res. Commun.*, **60**, 1309; 1974) have shown that the dioxygen stretching frequency in the cobalt(II)porphyrin analogue of haemoglobin is $1,105\text{ cm}^{-1}$. Now the recent report from Collman, *et al.*, puts values of $1,153$ and $1,150\text{ cm}^{-1}$ to the cobalt 'picket-fence' analogues with different axial bases. The change of the metal ion has not significantly altered the stretching frequency. But since the cobalt analogues possess one more d-electron than the iron compounds an EPR signal can be obtained. It was shown by Hoffman and Petering (*Proc. natn. Acad. Sci. U.S.A.*, **67**, 637; 1970) that the cobalt-oxygen adduct gives a signal with no cobalt nuclear hyperfine structure visible. They therefore concluded that the odd electron is largely on the dioxygen and that the formulation Co(III)-O_2^- is the most appropriate. In view of the similarities between the stretching frequencies of the dioxygen when bound to cobalt or iron model porphyrins and haemoproteins it is difficult to resist the conclusion that J. J. Weiss was correct in 1964.

In their oxygen-binding properties the picket fence porphyrins model the haemoproteins well. An interesting difference arises, however, in their very different affinities for the ligand carbon monoxide. The picket fence model binds CO irreversibly whereas the oxygen-carrying haemoproteins have considerably lower binding affinities which vary in the different mutant forms, in other words, as a function of protein structure. Collman *et al.* have now pointed out that the CO stretching frequency drops as the CO affinity of the haem group drops. It is suggested that the CO affinity in the protein is lowered as a result of the steric constraints of the haem pocket's causing the Fe-CO group to become bent and the iron-carbonyl bond to be weakened. Because the Fe-O-O structure is intrinsically bent its affinity is not correspondingly reduced. This ability to discriminate against CO binding may be of crucial importance since the biological catabolism of haemoproteins is a primary endogenous source of CO. The authors conclude that poisoning by CO of both haemoglobin and myoglobin would be considerably greater if the CO ligand were not forced to tilt.

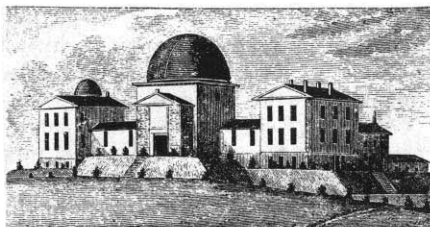
It is admitted, however, that it may be an oversimplification to ascribe all the differences between CO and O_2

binding to the steric constraints of the haem pocket. In planar systems with trans-axial ligands there is an appreciable electronic effect transmitted across the metal plane. This was nicely shown in model studies using cyano-cobalamin, which contains the cobalt(III)-corrin ring system. The infrared stretching frequency of the bound CN^- ligand was measured as a function of the ligand *trans* to it (Firth *et al.*, *J. Chem. Soc. A*, 2428; 1968). There is a perfect correlation between the binding constant of the cyanide group and its infrared frequency; the frequency drops as the binding constant drops. The decrease in affinity for cyanide is brought about by a change in the *trans*-axial ligand and can only be due to an electronic effect transmitted by

way of the metal ion. Now it is known that in the haem models Fe(deuterioporphyrin) (2Me-Im) the CO is bound 200 times less well than in Fe (deuterioporphyrin) (Im). 2-methylimidazole (2Me-Im) is a sterically hindered base which cannot approach the porphyrin plane closely (Rougee and Brault, *Biochemistry*, **14**, 4100; 1975).

Thus there is also a pronounced electronic *trans* effect in the iron-porphyrin system. Another function of the protein in the oxygen-carrying proteins could well be to control the distance between the haem plane and the bound histidine residue, thereby affecting affinities for *trans* ligands. It is not yet known whether such control will affect the affinities for both CO and oxygen in a parallel manner. □

A hundred years ago



CAMBRIDGE (U.S.) OBSERVATORY

A look into the earlier annals of the observatory of Harvard repays the inquirer at the outset by revealing the interest in astronomical pursuits which was felt in the old Bay State many years before the founding of an observatory was practicable in the United States. In 1761 the *Province* sloop was fitted out at the public expense to convey a Harvard professor, Winthrop, to Newfoundland, to observe the transit of Venus of that year; and in the troublous times of 1780 the old "Board of War" fitted out the *Lincoln* galley to convey Prof. Williams and a party of students to Penobscot to observe a solar eclipse. At so early a day was New England disposed to encourage scientific observations.

A new issue now arose. The sudden appearance of the splendid comet of 1843 was, happily, the occasion of final success in the founding of the present institution. Cambridge was immediately appealed to for information about this strange comet. But the observers had no parallactic instruments or micrometers of the least value for its observation. While they were endeavouring to obtain data to compute the comet's orbit, a meeting of citizens was held, under the sanction of the American Academy, to take measures for procuring a first-class equatorial; the needed amount of \$20,000 for the instrument was contributed in Boston, Salem, New Bedford, and Nantucket. The equatorial was ordered from Merz and Mahler, of Munich, and Harvard determined to erect a new observatory. The location selected was 80 ft. above tide-water, and 50 ft. above the plain where the soil was found favourable for the stability of piers for the instruments. In 1844 the buildings were occupied, and an equatorial of 44 in. focal length and $2\frac{1}{2}$ in. aperture, and

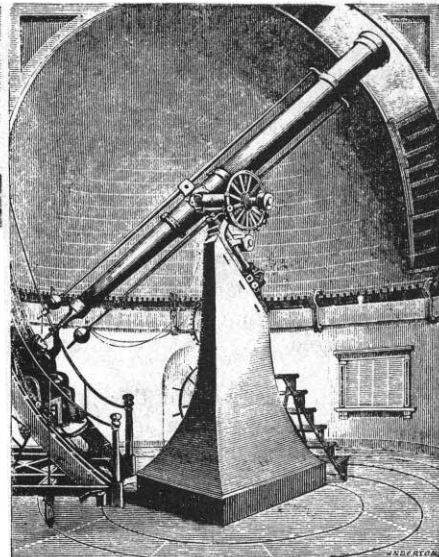


FIG. 2.—Cambridge Equatorial.

a transit instrument loaned by the United States, were temporarily mounted for observations until the arrival of the great refractor. This was placed in position June 24, 1847.

The great equatorial, made in 1847 by Merz and Mahler, of Munich, has an object-glass of 15 in. in diameter, and a focal length of 22 ft. 6 in. The power to its eye-piece ranges from 100 to 2,000; the hour-circle is 18 in. in diameter. The movable portion of the well-balanced instrument is estimated at three tons. Its original cost was about \$20,000. The sidereal motion given to this telescope is now secured by clockwork from Alvan Clark, which is spoken of by the observers as the only known "driving-clock working with perfect steadiness." The telescope rests on a central granite pier, in constructing which 500 tons of granite were used. It is 40 ft. high, and rests on a wide foundation of grouting 26 ft. below the ground surface. Upon the top of the pier is laid a circular cap-stone 10 ft. in diameter, on which is the granite block, 10 ft. high, bearing the metallic bed-plate. This instrument is in the central "Sears Tower."

from *Nature*, **15**, January 4, 201; 1877.

articles

Stonehenge I and lunar dynamics

A. D. Beach

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The concept of Stonehenge I as an accurate predictor of eclipses can be criticised on the grounds of inadequate short-term precision, unnecessarily good long-term tracking of the lunar nodes, and irrelevance to cultural needs. A more practical application of the astronomical characteristics of the structure is that of lunar apogee tracking, which would allow prediction of minimum tidal disturbance to cultural communication across the North Sea and English Channel.

FOLLOWING Hawkins' numerical analyses and discussion of the alignments between components of the Stonehenge monument on Salisbury Plain, England, (described by Atkinson²) the succeeding decade of investigation has further emphasised the dichotomy of viewpoints between the astronomers and the archaeologists. Some of the original deductions of Hawkins have been strongly criticised by both groups³⁻⁶, but the work of Hoyle⁷ and of Newham⁸ has extended other aspects and shown that Stonehenge I, the earliest phase of the megalith, could have been used as a universal eclipse predictor, independently of any eclipse cycle.

Operation of the device in this mode would have been simple, but the sophisticated intellectual reasoning needed to design the system conflicts to a high degree with the otherwise satisfactory archaeological picture of Britain as having a technologically primitive agricultural economy. Although now endorsing an astronomy-oriented content in the Stonehenge structure, most archaeologists resile from the 'eclipse computer' concept *per se*, because of this incompatibility, preferring religious connotations to explain the interest in the Sun and Moon⁹.

Stonehenge I

The consensus of opinion is that the initial phase of Stonehenge consisted of:

- (1) The raised bank and quarry/ditch (both originally about 2 m from datum).
- (2) The circle of 56 pits, named 'Aubrey holes' (A holes), filled in shortly after their original excavation. Erosion of the surface since the construction date makes their original form doubtful, but it is noteworthy that the excavated material, replaced in the hole, would exceed the pit volume and form a chalk mound, an ideal quasi-permanent marker of high visibility in twilight conditions. The name Aubrey holes may be rather misleading.
- (3) The primary alignment, represented by a line from the central point to the heelstone⁹, with its associated quadruplet of equally spaced 'A' postholes. This is not the axis, which is of phase II/III origin.
- (4) The 'causeway' postholes at the 'entrance'⁸.
- (5) The locations of the 'station stones'. The actual station stones appear to have phase III characteristics, but the

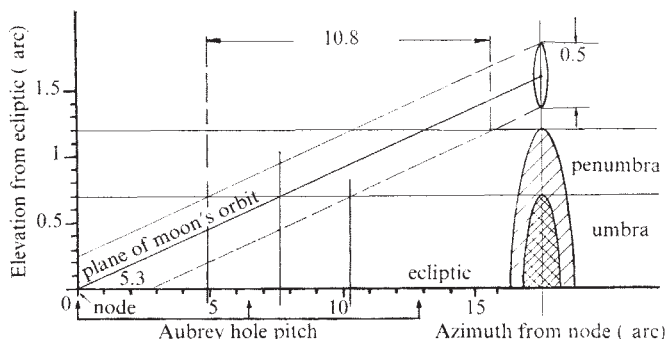
locations are deemed significant, and relevant to phase I because of their near-rectangular geometry resulting from the choice of latitude for the Stonehenge site⁹.

Angular resolution and the Aubrey holes

It is a weak point in the astronomical argument that, as an eclipse predictor, Stonehenge I is embarrassingly comprehensive, potentially capable of predicting all eclipses, when only about half could be observed from a single location. A much more stringent criticism, however, is the inadequacy of the moment-to-moment accuracy of the tracker markers in the 56-step 'plan position indicator' scheme of Hoyle. An unsynchronised incremental movement of 6.43° in each of three markers, Sun, Moon, and node-pair, gives a potential average angular error of $\sqrt{3}$ Aubrey holes, or more than 11° arc. The Lunar eclipse prediction requirement can be assessed from Fig. 1 and it can be seen that an 11° error would be sufficient completely to miss even an umbral eclipse if it were tangential. Moreover, solar eclipses require better than twice the resolution needed for lunar eclipse prediction. At any time in the operation of Stonehenge I, the average error could have been halved by a simple doubling of the number of Aubrey holes and of the rates of marker movement, so it is reasonable to suspect that eclipse prediction was not the prime purpose of the Stonehenge I Aubrey hole array.

The number 56 of Aubrey holes is of critical importance in the circumstantial evidence for the astronomical case. Fifty-six is very close to three times the nodal regression period in years (55.835), and has led most investigators to accept the relevance of the extraordinary accuracy of node tracking implicit in this close match. Node markers moved around the Aubrey circle at three Aubrey holes per year (A.H. yr⁻¹ hereafter) relative to a fixed 'equinox', accumulate an error of only one Aubrey hole in more than a century, and can thus easily be reset by 93 yr periodic

Fig. 1 The angular relationships between mean lunar angular diameter and the Earth's umbra and penumbra, in a lunar eclipse situation. The incremental angular resolution of the Aubrey hole array would not be satisfactory for a definitive prediction of an eclipse, if the average error were $\sqrt{3}$ Aubrey holes (see text).



recalibration observations of maximum northerly midwinter full moonrise azimuth. The figure of 93 yr is arrived at because five times the nodal regression period is 93.06 yr, adequately close to an integral number of midwinter solstices.

The logic of this interpretation of the significance of the number 56, however, is suspect. The argument of Hoyle is that the most northerly azimuth of midwinter fullmoonrise (the peak of the nodal cycle), was determined by interpolation, using the A posts (and thus achieving independence from a rather infrequent coincidence of fullmoonrise with winter solstice) primarily for the purpose of determining the date of that event, the reset moment of the nodes markers. But it is a simple fact that the cancellation of any accumulated error in the nodes markers could have been achieved many times per decade, by observation of lunar eclipses. Any total lunar eclipse of long duration would automatically define the near-zero angular displacement of the nodes from the Sun–Earth–full moon axis to a precision rather superior to that achievable by deducing the date of maximum northerly virtual fullmoonrise (abbreviated hereafter to MNVF).

The concept of the interpolation technique is valid, provides an elegant solution to the use of the A postholes, and is highly relevant to the argument that follows, but the task assigned to it by Hoyle cannot be considered logically justified.

The new dating

The radiocarbon dating revolution within archaeology has caused a considerable reassessment of the process of cultural diffusion from Western Asia to Europe, and of the relation of Stonehenge to the Wessex culture. Following the publication by Suess¹⁰ of his dendrochronologically-based calibration chart for radiocarbon samples, Stonehenge I in particular has become very difficult to assess. The most relevant sample, I-2328, chosen as representing the earliest construction phase, is from the antler pick found 3 in above the bottom of the outer ditch at 6 ft depth, in the primary silt⁹. Isotopes Inc. dated this at 2180 ± 105 b.c.

The present position is summarised in Fig. 2. Because of

the 'staircase' curve, the new calibration would give essentially the same radiocarbon date for samples of real origin dates between about 2500 bc and 3000 bc. (The reality of the Suess calibration curve retrogressions has of course been questioned, but the larger ones, such as those in Fig. 2, do seem to be caused by an actual fluctuation in the ^{14}C levels of the period^{11,12}.) Further sample dating is urgently needed.

The anomaly of Stonehenge I is now better realised. The uncertainty of dating does not obscure the probability that this geometrically established structure predates the main building phase of the Egyptian pyramids, and that it may have been contemporary with the proto-literate Sumerians¹³.

An important outcome of the new dating is the modification now necessary to the values of parameters used to calculate the positions of some astronomical entities. It will be seen that the date regime 3000–2500 bc is of great significance to an improved understanding of Stonehenge I.

Static geometry and dynamical astronomy

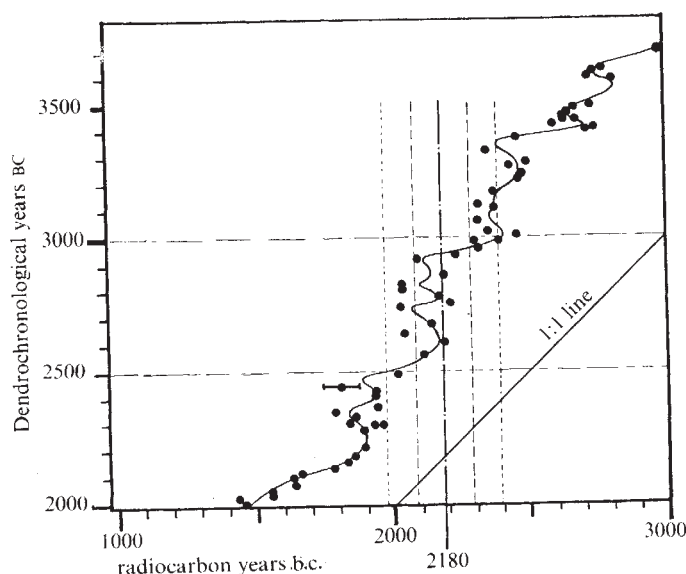
In the past the emphasis has been on the midsummer sunrise phenomena at Stonehenge. For this to have obscured for so long the much better match of the structural geometry to the midwinter moonrise phenomena, is rather surprising. The entrance includes the range of moonrise azimuths from that represented by the heelstone, to the MNVF azimuth, and is asymmetric with respect to the avenue. This same range of azimuths covers the causeway postholes, investigated by Newham, all of which are northward of the heelstone azimuth and which have three ranks terminating close to the MNVF azimuth.

This emphasis on the extreme northerly azimuth of moonrise is evidently very important to investigations of the motivation behind Stonehenge I, and now that a more definitive range of radiocarbon dates has been derived for this part of the structure, it is relevant to attempt the determination of the actual solstitial moonrise azimuths for that period, so as to achieve a greater understanding of the designer's motivation.

I have used the polynomial expressions for the angular positions of features of the lunar orbit, given in the explanatory supplement to *The Astronomical Ephemeris* (HMSO, annually), to examine the moonrise azimuth situation in the period 3500–2000 bc. When using these expressions it must be remembered that the imprecisely known secular acceleration of the Moon leads to some uncertainty regarding the lunar longitude at times remote from the AD 1900 epoch, but it is considered that the expressions for the lunar node and perigee are accurate to a few degrees for periods up to 100 Julian centuries (Her Majesty's Nautical Almanac Office, personal communication). The parameter T (Julian centuries) is common to all the polynomial expressions, and its value for each winter solstice of the relevant years can be determined by solving for T the solar longitude expression when the longitude is defined at 270° . The accurate value for T can then be inserted into the expressions for obliquity of the ecliptic, and for the longitudes of the Moon's ascending node and perigee.

The spherical trigonometry used to determine from these parameters the azimuth of virtual moonrise has been described elsewhere⁷ (moonrise is defined as the tangency of the Moon's lower limb to the horizon). Assuming the present-day value for the eccentricity of the lunar orbit, and that the atmospheric conditions were 1002 mbar and 10°C , as used by Hawkins¹, then with the measured value of 0.60° for the elevation of the relevant portion of the Stonehenge horizon, the virtual moonrise azimuth can be determined to a high accuracy (typical uncertainty for ± 50 mbar in atmospheric pressure, for example, would be $\pm 0.05^\circ$ arc in azimuth).

Fig. 2 The Suess dendrochronologically-based radiocarbon calibration curve for the calendrical interval 2000–3500 bc (adapted from ref. 10), with the radiocarbon age data for Stonehenge I sample I-2328 (2180 ± 105 bc) superimposed. The vertical dashed lines define the normal distribution confidence levels of 68% and 95%. Suess' typical dating uncertainty limits are given for one of his points; the limits for the other points have been omitted for clarity.



Coincidence of azimuths

The conditions for extreme northerly virtual fullmoonrise are that the line of nodes be exactly in quadrature at the winter solstice (the longitude of the ascending node being zero), and that apogee be at the virtual fullmoon point, for minimum horizontal parallax. The first criterion alone is a moderately rare phenomenon, even when allowing a few degrees of longitude tolerance. Within the arbitrary limits of ± 1 A.H. of longitude, eligible coincidences occur approximately three times every 93 yr. Because it is at this time in Hoyle's scheme, that the nodes marker-pair is checked for 'longitude' accuracy, however, it is highly significant that the normal incremental angular resolution of 1 A.H. is much improved by a knowledge of the temporal mismatch between nodal quadrature and winter solstice.

For the second criterion to coincide with the first is rather more rare. To an arbitrary limit of 2 Aubrey holes of longitude accuracy (for a hypothetical apogee marker), coincidence occurs at intervals of about 1247 yr (67 cycles of nodal regression and 141 cycles of apogee precession).

A computer programmed to perform the necessary search, showed that at the winter solstice of 2871 BC ($T = -47.699$ Julian centuries from AD 1900), the ascending node of the lunar orbit had a longitude of about 1° , and apogee was about 7.8° from the virtual full moon point. Figure 3 shows the sequence of MNVF azimuths between 3250 BC and 2400 BC. Of particular interest is the sequence of apogee MNVF azimuths for the years 3057, 2871 and 2685 BC. At 41.8° , 41.83° and 41.88° , they are very close to the extreme limiting azimuths possible at those times.

It is remarkable that the causeway postholes investigated by Newham⁸ contain three ranks terminating at azimuths which he measured at 41.8° , 41.9° and 41.9° .

Fig. 3 Computed azimuths of virtual fullmoonrisings at winter solstice, in the calendar date regime 2650 BC to 3150 BC, as seen on the NE horizon of Stonehenge, when the ascending node of the lunar orbit was less than one Aubrey hole pitch from the equinox. The pair of nearly vertical dashed lines are the azimuth limits for apogee and perigee when the longitude of the ascending node is zero. The rare sequence of nearly perfect apogee and perigee azimuths, which occurred in the Stonehenge I period, is shown emphasised.

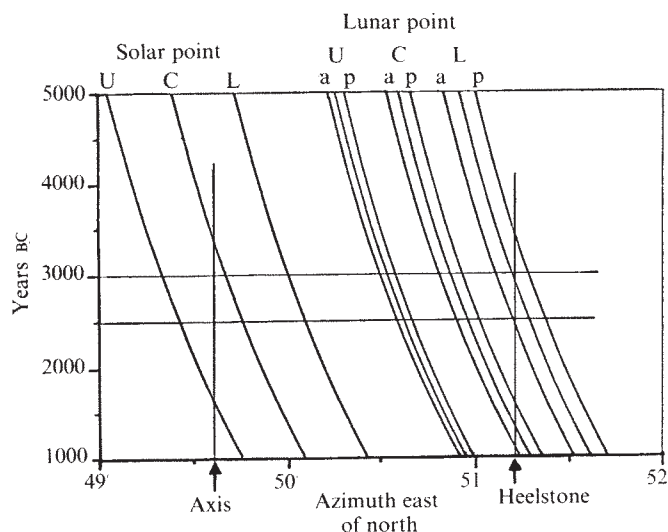
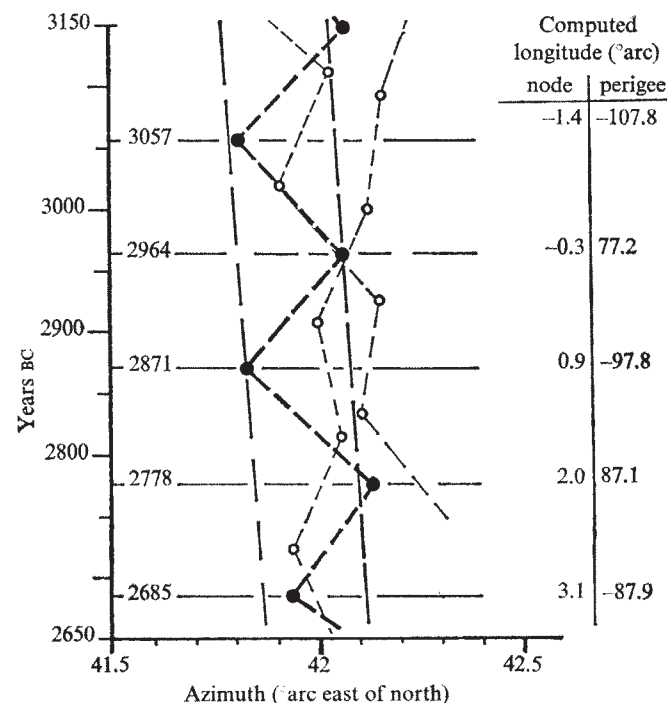


Fig. 4 The heelstone azimuth. The curves show the rising azimuths for the upper, (U), and lower, (L), limbs, and for the geometrical centre, (C), at winter solstice and nodal syzygy for the Moon, and at summer solstice for the Sun. Apogee and perigee displacements are given for the Moon. The general shift of all azimuths with time is due to the changing obliquity of the ecliptic. The 2500 and 3000 BC time lines facilitate correlation with Fig. 2. The axis azimuth is approximate only, but the heelstone azimuth was accurately measured by Hawkins¹, as 51.222° . At the winter solstice of the year 2857 BC, the ascending node of the lunar orbit had a computed longitude of 90.0° (syzygy) and the azimuth of fullmoonrise for the lower point was computed to be 51.28° .

Such a close coincidence encouraged me to pursue the investigation and examine the remaining feature of Stonehenge I at the other extreme of the entrance azimuths, the heelstone. Robinson⁹ had pointed out that the heelstone was a better marker of the node at winter solstitial syzygy than it was of midsummer sunrise, and my computations bear this out to a remarkable degree. Figure 4 demonstrates the striking match of the heelstone azimuth to that of the moonrise azimuth for nodal syzygy in the date regime centred on 3000 BC (again for lower limb tangent to the horizon).

For the sinusoidal function of winter solstitial moonrise azimuth to be marked in the Stonehenge I structure at no less than four points (the rare triple extreme event and the 'crossover' point) is prime evidence for a knowledge of the existence and of the accurate position of the lunar node, that can be described only as astonishing in its precision.

Moreover, the marking of the apogee MNVF azimuths and the omission of the perigee equivalents has some interesting connotations.

Apogee tracking

In Hoyle's scheme, the most accurate tracking is that of the nodes, which in 2871 BC accumulated an error of 1 A.H. every 111 yr (when moved at 3 A.H. yr⁻¹ relative to a fixed 'equinox'). This figure is arrived at by differentiating the polynomial expression for the longitude of the lunar node, with respect to time, and inserting the appropriate value of T into the resulting expression, to obtain the real regression rate (18.6107 tropical years per rev.) and then taking the reciprocal of the difference between the ideal Aubrey hole marker rate (3.00902 A.H. yr⁻¹) and the prescribed rate.

Remarkable though this accuracy (or coincidence) seems, it is outweighed by an even more accurate relationship. Performing the same calculation for an apogee marker, the precession period is found to be 8.84516

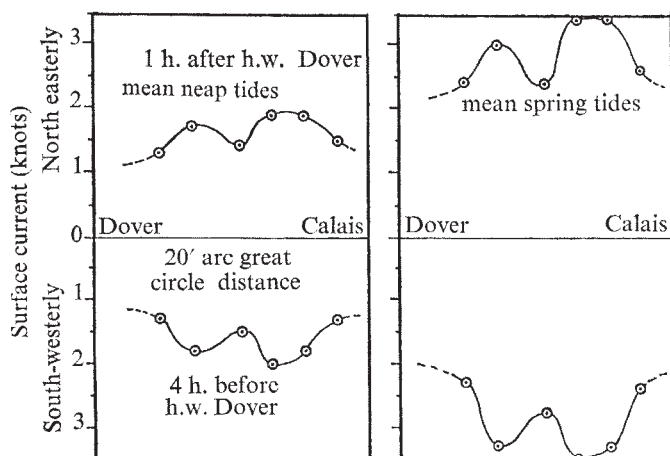


Fig. 5 Present-day surface currents in the Dover Strait. By rowboat, at 2.5 knots, the 20' arc surface traverse would require 8 h if there were no tidal disturbance. For a daylight journey, from embarkation to landfall, the most suitable tidal conditions would be those at apogee neaps, when the peak surface current may be expected to be about 13% less than at mean neaps.

tropical years, the ideal A.H. tracking rate 6.33115 yr^{-1} , and the error accumulation rate, for a marker moved at $6.33333 \text{ A.H. yr}^{-1}$ (6, 6, and 7 Aubrey holes in a repeating triennial sequence), 1 Aubrey hole every 458 yr. Only for 56 Aubrey holes does this combined accuracy exist for both nodes and apsides trackers.

Such circumstantial evidence has been the basis for all previous astronomical interpretations of Stonehenge, especially the eclipse predictor aspect mentioned above, but it is also true that the presumed cultural motivation for Stonehenge I has been specified to match these hypotheses. Since eclipses could have served no practical purpose in what was essentially a subsistence farming economy, the motivation has been stated as 'religious'; surely the lowest common denominator of imprecision and evasion.

The hypothesis discussed below is an attempt to demonstrate a strong cultural motivation for the origin of Stonehenge I, in which the observations of natural phenomena are logically extended, and lead to the analogue device which has for so long puzzled a multitude of investigators. The only assumption that need be made is that the spectrum of intelligence present in the indigenous population of Neolithic Britain and Europe, was essentially the same as it is today, surely an acceptable criterion in view of the precision of azimuthal delimiting described above.

The relevance of tides

Thom^{14,15} has suggested that the background to the north British megaliths could have been an interest in the lunar influence of tides, but these structures he dated from astronomical considerations, to about 1750 BC. Nevertheless, there may be a connection with the 1,000 yr older Stonehenge I lunar observatory.

The pre-Wessex south British cultures seem to have been culturally isolated from mainland Europe during the Stonehenge I period. A major factor contributing to this situation may have been the difficulty of traversing the strongly tidal English Channel, Dover Straits, and North Sea. To the operator of any surface craft whose speed is limited by the use of sails or oars, the need for tidal prediction is obvious, even today. In 2750 BC with sea levels averaging 4 m lower than at present¹⁶, and tidal currents over the continental shelf therefore appreciably more severe, and with the surface craft operational qualities perhaps less adequate than those of modern craft, the situation could have been even more relevant.

Figure 5 has been derived from a standard tidal stream atlas¹⁷, and shows the present-day strength of the tidal streams in the Dover Strait. The high values of stream velocity over the entire width of the Strait emphasise the need for small craft to choose conditions of minimum tidal disturbance for a safe and reliable crossing.

Consideration of the criteria determining tidal phenomena shows that the maximum tide-raising force exists when the following priority-ordered conditions are met simultaneously: (1) Lunar phase 'New' or 'Full', with an eclipse as the extreme event; (2) Moon at perigee; (3) Earth at perihelion (2 and 3 because of the inverse-cube law of tide-raising force with separation distance); (4) Earth at equinox, for peak semi-diurnal tide; (5) Line of nodes in syzygy. Condition (1) is virtually the defining parameter of 'spring tide', with (2) as a major modulator of the effect, having a force ratio, perigee-apogee, of 1.39. The force-ratio for (3) is 1.126. Condition (4) is important since the Atlantic Ocean responds mainly to the semi-diurnal tide-raising force¹⁸. Least important is (5), serving only to reduce to zero the very small effect of a few degrees separation of the solar and lunar radius vectors.

With the lunar perigee-apogee situation known to have existed at Stonehenge in the period 3057–2685 BC, Neolithic observers had a significant opportunity to notice that the difference in spring tide range correlated with the observed change in MNVF azimuth, and thus to link these two phenomena. But for this to be fortuitous is of far too low a probability to be taken seriously. Some link between the variation in tide range and the lunar motion, must have been suspected for the motivation to be established to carry out detailed observations as evidently occurred at Stonehenge.

The first order effect of condition 1 is obvious enough, but the modulation due to (2) might seem rather more difficult to observe. There is however, a curious near-commensurability in the relevant intervals which immensely aids observation. Expressed in terms of the lunar motion:

Synodic month (phase to phase):

$$29.53089 \text{ d} \times 14 = 413.428 \text{ d}$$

Anomalistic month (perigee to perigee):

$$27.554551 \text{ d} \times 15 = 413.318 \text{ d}$$

$$\text{Difference} = 0.11 \text{ d}$$

Hence the perigee/apogee axis moves through one lunar phase cycle in slightly less than a 14-lunation period. In terms of the tide-raising force, therefore, there is evidently a periodic modulation in the range of the Spring tide, and of the strength of the tidal currents, occurring cyclically over 14 lunations, with maxima at 7-lunation intervals, alternately at New and Full Moon. Similarly the neap tide effects vary with the same periodicity, with minima at every seventh first and third quarters.

If this 14-lunation periodic effect had been exact, then possibly no problem would have existed, but the slow phase shift between the incommensurable periods of lunar phase change and perigee precession would destroy the relationship in less than a century. The motivation to examine, in a more detailed fashion, the motion of the Moon, may well have stemmed from this inexactitude.

Calibration of the perigee-apogee markers

Numerical coincidence may, once again, be cited, but it is a fact that $56 = 4 \times 14$. The convenience of the Aubrey hole number allows the existence of a perigee/apogee marker-pair which could be moved at the simple prescription rate of 4 A.H. per lunation relative to the Sun marker. In Hoyle's tracking scheme, the Sun marker moves at the observationally corrected rate of 1 A.H. per 6.52218 d or 4.527716 A.H. per lunation. The net perigee marker movement rate is thus 0.527716 A.H. per lunation in the same direction as the Sun and Moon markers. This is

equal to 6.5269 A.H. yr⁻¹, differing from the computed ideal rate of 6.3309 A.H. yr⁻¹ by 0.1960 A.H. yr⁻¹.

If the Apogee marker were set into operation at the winter solstice of 3057 BC or 2871 BC, at this empirically determined rate, then 93 years later the perigee marker would be 18 Aubrey holes ahead of its proper position, after 10½ revolutions around the Aubrey circle. To assess the correct rate should have been a simple task to the geometers who designed the Stonehenge I structure.

Location and planning

Why Stonehenge? Or rather, why Salisbury Plain? As a site for a tide predictor it could hardly have been selected much further from the coastlines of what is now Southern England. But Stonehenge is not misplaced if consideration is given to the observational requirements. These may be listed as:

- (1) Elevated plateau for an unobstructed view of the horizon.
- (2) Level and treeless horizon, at least over the appropriate range of azimuths, so that the simplest relationship exists in the azimuth sequence.
- (3) Horizon slightly elevated and not too distant, to avoid the worst effects of atmospheric refraction and absorption.
- (4) Nearby land sloping slightly downwards away from the observing site to drain away winter groundmist, again at least over the appropriate azimuths.
- (5) Not in the vicinity of a seacoast or of a large body of water, to avoid seamist or fog in winter.

That the Stonehenge site meets all these conditions may be an indication of the geographical coverage available to the designers. The Station Stones also indicate this, if their near-rectangular geometrical relationship really is due to a choice of latitude. The cultural aspects of these points have not been adequately discussed: the implication is of a cultural homogeneity of hitherto unsuspected extent.

The planning of the measurements and the choice of site certainly seem to have been justified by the practical execution of the observations. It now seems very likely that the causeway postholes represent, as Newham suggested, a sequence of measurements now that can be seen

to culminate in the apogee moonrise azimuths of the winter solstices of 3057, 2871 and 2685 BC. The chance of accidental coincidence of azimuth, of the triple extreme astronomical event, and of the terminal postholes is slender indeed.

The ability to track such an elusive entity as the lunar apogee can be logically derived from the circumstantial evidence of Stonehenge I, as has been argued earlier. The later argument, of tidal prediction, is wholly speculative, being on a par with the eclipse prediction hypotheses, but contains the redeeming feature of practical motivation, unconnected with 'religion'. Moreover, the practical application is of a lower order of intellectual achievement than is the prodigious feat of designing and actually using a Neolithic lunar observatory. It is the latter for which we have evidence, and speculation on its purpose seems to me valid if there is required no great extension of the talents so clearly evident, five millennia after their application.

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Mantle origin of Cordilleran granites

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'Granite' batholiths of recent destructive margins have strontium isotopes inconsistent with crustal fusion. Associated enormous crustal thicknesses are produced without compressive shortening. These features necessitate accretion by vertical addition of predominantly mantle-derived calcalkaline intrusive and extrusive magmas.

ONE of the more controversial debates in contemporary earth science concerns the origin of granitic rocks, particularly those batholithic volumes characteristic of modern ocean-continent convergence zones. For some time the consensus of opinion was swayed by the weight of experimental evidence^{1–3}, accumulated over two decades, which shows that batholiths are derivable by partial melting at crustal pressures provided an ample source of meta-sediments is available. The overlap of granite melting conditions with granulite facies metamorphism of lower crustal regions is consistent with the extraction of such

partial melts from their residua⁴ leading to the two-layer crust typical of continents.

Theories of granite melting in the crust have been modified to explain the observed time-composition relations in batholiths which characteristically vary from early intermediate through to acid members of the granite family at later stages^{5–7}. Detailed mapping in the Mesozoic and Cenozoic batholiths of Peru⁸ has shown that minor cycles of this type, due to high level fractionation⁹, are superimposed on a major cycle of primary differentiation starting with gabbro and terminating some 80 Myr later with adamellite. Considering the time span occupied by this primary cycle (note also California^{6,10} and Alaska¹¹) it is preferable to invoke a mechanism whereby separate batches of magma are generated by partial melting at successively higher crustal levels¹². This conclusion follows from the crustal pressure experimental data: more acidic (and hydrous) melts are produced at lower pressures and temperatures. In the context of crustal melting, the most plausible way of having the highest melting temperatures

recorded by the earliest intrusives is to suppose that crust-mantle interaction occurs at deep levels. Thus the origin of Cordilleran granites became linked to mantle processes (see also refs 7, 13-15) and I suggested¹² that rising andesitic magmas, generated within or above a subduction zone and either underplating or penetrating the crust, supplied essential heat and volatiles necessary to initiate granite melting at the deepest crustal levels. Once initiated, the granite cycle would be perpetuated with progressively more acidic contributions from shallower levels as the crust warms through from the base. Through this rather intimate association of potentially extrusive and intrusive magmas the time and space patterns of the latter are reconcilable, with a crustal anatectic origin. It follows that this evidence, and that involving time-composition relations, does not necessarily imply a mantle source for Cordilleran granite magmas which is where the strength of earlier arguments of this nature lay (ref. 14, for example). However, as field data and geochemical-isotopic observations have become more numerous, there have emerged two other persuasive lines of reasoning, considered below, which apparently place granite magma genesis at mantle depths.

The fractionation of large volumes of basic magma to produce granite batholith magmas¹³ is not a popular theory because of the proportionately larger volumes of intermediate fractionates which are not found. Instead, these days the question we ask is whether relatively small percentages of basaltic to ultrabasic mantle melting or rather greater degrees of intermediate composition lower crust melting is responsible for granite magmatism.

Evidence from strontium isotope ratios

First we have the low initial strontium isotope ratios ($^{87}\text{Sr}/^{86}\text{Sr}$) which are emerging as a ubiquitous feature of Cordilleran granites. The values measured on a wide range of Western American granites range from 0.703 to 0.708 with the majority around 0.706 (refs 16-20), and values have been observed to increase with decreasing age in some Peruvian intrusives²¹. These ratios are a little higher than values being produced direct from the mantle in regions devoid of continental crust—a maximum present-day ratio of 0.704 in the case of Pacific oceanic basalts²². However, they are very much lower than contemporary values in crustal anatectic source regions containing ancient sialic basement or its erosional products. Values between 0.71 and 0.78, appropriate for such regions^{8,16,23} depending on age and Rb/Sr ratio, seem to preclude an origin by direct crustal melting as described above.

Before accepting this as a rigorous conclusion, experience with strontium isotopes suggests we should beware of several ways in which the crustal melting hypothesis might be rescued. For example, guided by oxygen isotope work in the British Tertiary igneous province²⁴, it has become fashionable to reassess isotope patterns in terms of hydrothermal strontium transfer between intrusive and intruded rocks. It might be suggested that measured granite samples, usually taken from the exposed upper regions of batholiths, have acquired artificially low ratios due to equilibration with their mantle-derived andesite cover. However, in view of so many similar values having been determined from many different regions and erosion levels, I doubt that such an equilibration process is particularly important except in a local sense. The validity of strontium isotope interpretations can be checked by looking for ^{18}O depletions inherited from hydrothermal alteration²⁵, and the case for making parallel isotope measurements of strontium and oxygen in future work of this type is becoming recognised.

Perhaps a more significant method of producing low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios by crustal fusion would be if the deep source region had a low Rb/Sr ratio. There is little disagreement about strontium concentrations in the continental crust and an average value of about 375 p.p.m. seems

appropriate²⁶. Crustal rubidium concentrations are more widely debated^{26,27}, but values close to the average andesite²⁸ (about 40 p.p.m.) seem appropriate and yield an average Rb/Sr ratio near 0.1. Bearing in mind the strong upward crustal concentration of rubidium²⁹, the incrementation rate of $^{87}\text{Sr}/^{86}\text{Sr}$ in buried crystalline basement might be less than 0.004 in 10^9 yr (corresponding to Rb/Sr=0.1). It follows that ratios normally ascribed to mantle could be derived from a relatively old lower crustal source region of low Rb/Sr. Of course, this incrementation rate is rather low in comparison to the mature $^{87}\text{Sr}/^{86}\text{Sr}$ ratios quoted earlier for exposed basement rocks, supposedly equivalent to those buried beneath younger orogenic belts, whose anatectic products would be distinguished easily.

At present this dilemma is not fully resolved but the temptation is to suggest that, since the Rb-poor basement rocks are usually in refractory granulite facies, the production of large volumes of low $^{87}\text{Sr}/^{86}\text{Sr}$ ratio granites from them seems most unlikely. This begs the question of how such rocks in the basement became deficient in rubidium and here it seems difficult to escape extraction by a melt phase leaving strontium partitioned in high temperature mineral residua³⁰ though this melt need not be indiginous and could be derived largely from the mantle. Shallow zones of unaffected old crystalline rocks, such as exposed in coastal Peru⁸, would continue to accumulate radiogenic strontium by retaining their rubidium.

A final possibility is that underplating of the lower continental crust by low temperature, hydrous low $^{87}\text{Sr}/^{86}\text{Sr}$ mantle magmas, rapidly encountering their solidus temperatures, yields an immature zone later available for further partial melting (see refs 12, 14, 31). This merely splits granite magma generation into a two-stage process virtually indistinguishable from a direct mantle source on the grounds of strontium isotopes.

On balance, the conclusion to be drawn from strontium isotope observations is that Cordilleran granites contain a prominent component of recent mantle derivation. The style of granitic activity in relation to tectonic processes has doubtless changed considerably over the Earth's history^{32,33}, but voluminous granitic rocks generally record the isotopic imprint of recent mantle derivation from the early Precambrian³⁴, though fossilised early Phanerozoic Cordillera (for example Caledonian younger^{35,36} but not necessarily older³⁷ granites), right up to recent times¹⁶⁻²¹. Lead isotopes provide further corroboration in that such data as are available indicate derivation of young granite and older granite gneiss leads direct from primary mantle growth curves^{38,39}.

Crustal thickness, deformation and accretion rates

Now let us examine a second line of attack on crustal melting hypotheses. From seismic and gravity data (refs 40 and 41, for example) it is well established that continental crustal thicknesses at destructive margins are much greater than average. In the central Andes, for instance, the crust reaches a maximum of 70 km thickness beneath the volcanic Cordillera⁴¹, whereas 35 km is more typical of inactive regions. How does the crust locally acquire this great thickness? There are two possibilities: (1) crustal shortening whereby material is piled up due to laterally compressive forces produced where two plates are colliding and (2) thickening due to the addition of material vertically from the underlying mantle. Unlike continent-continent convergence zones, such as the Alps and Himalayas where strong compressive folding and overthrusting are in evidence⁴³, the field data from active ocean-continent boundaries, the sites of Cordilleran batholiths, reveal a striking lack of compressive tectonics. The intrusion of the Peruvian granites apparently created a tensional stress regime opening fracture systems which were the sites of

block faulting, vertical movements⁴⁴ and even possibly crustal extension rather than shortening. The conclusion is that where two masses of like density converge the result is diastrophic whereas, at destructive margins, ocean crust and upper mantle are not forced down but dive quietly beneath continental crust with apparent ease.

Thus we have another significant difficulty for the crustal melting origin of granites. These rocks occupy about 25% of the crustal volume along destructive continental margins (average estimate based on various interpretations: refs 8, 41, 44–46) and, if we exclude thickening by crustal shortening, a large proportion of this material must be new crust recently derived by mantle melting. The other more positive candidates for derivation from the mantle are the extrusives, mainly andesites, but their volumes are too small to account for crustal thickening alone as they are essentially a veneer reaching only several km in thickness at maximum⁴⁷. These concepts are best illustrated by a simple numerical estimate of the modern rate of crustal evolution involving both contemporary intrusives and extrusives. As a type example we can use the young Peru Coastal Batholith region, for which we now have abundant data^{5,8,9,19,20,21,40,41,44,47}. The volume of crust produced by igneous events in this region during the last 10⁸ years can be estimated at $1.2 \times 10^6 \text{ km}^3$, based on a width of 60 km normal to the margin, a length of 1,000 km in Peru, and a material thickness of 20 km. The rate of crustal evolution from igneous activity in Peru is therefore about $0.012 \text{ km}^3 \text{ yr}^{-1}$ where “evolution” includes any recycled products of crustal fusion in addition to new material.

Now, in terms of crustal growth Peru was chosen because it probably represents an average accretion rate between the more dramatic effects of intracontinental mountain building, despite crustal shortening, and the lesser volumes involved in island arcs. To advance the argument we can estimate the contemporary crustal evolution rate at $0.012 \times 40,000/1,000 = 0.5 \text{ km}^3 \text{ yr}^{-1}$ as I am persuaded (R. Oxburgh, personal communication) that we should consider about 40,000 km length for the world's recently active subduction zones and recently developed intracontinental margins. Again, this rate would include any recycled crustal fusion products. Obviously there will have been some erosion over the time spanned, allowance for which would slightly increase the rate estimated above. However, the amount will be too small to be significant in this approximate calculation, especially as much of the eroded material is reconstituted as continental crust by uplift at intracontinental collisions or by accumulation on the continental shelf. (For example, the Atlantic bordering shelf regions receive a surprisingly large proportion of the Andean erosion products because of eastward drainage⁴⁸—relatively little sediment accumulates in the Peru–Chile trench⁴⁹.)

Accepting that crustal evolution is occurring at about $0.5 \text{ km}^3 \text{ yr}^{-1}$, it follows that if all this is new material added to the crust, in which case our evolution rate becomes the crustal growth rate, 10,000 Myr would be required to produce all $5 \times 10^6 \text{ km}^3$ of continental crust which now exist. Of course, it seems highly likely that accretion rates would have been rather higher in the past—a time-average of $1.1 \text{ km}^3 \text{ yr}^{-1}$ is required over the Earth's history¹²—and our modern figure of about half the time average is consistent with the decay of long-lived radioisotopes having reduced modern heat productivity to about a quarter of its initial value⁴⁹.

If we drop crustal growth rates to include only the extrusive component of destructive margins then a rate nearer $0.1 \text{ km}^3 \text{ yr}^{-1}$ would result, because intrusives predominate over extrusives. The observed crustal thickening at destructive margins could then not be achieved without severe shortening, and it is also difficult to see how continents could ever have reached their present volumes without

disproportionately rapid early accretion rates. Unfortunately, numerical estimates of accretion rates can never be better than highly approximate. Yet on balance, the evidence from thickening and crustal growth again demands that Cordilleran granite magmas be derived from mantle depths, whether this is by a direct melting and emplacement event or by the indirect two-stage process mentioned earlier.

Andesites, granites and mantle melting

If granite batholiths owe their origin to mantle melting, we should return to the experimental data to consider whether the observed time–space–composition relations can be produced in this revised context. In order to produce magmas over the entire lifespan of a destructive margin it is necessary to continuously replenish the source region or igneous activity would have limited life. Some involvement of subduction zone materials is implied either as a source of melt or of volatiles capable of melting overlying mantle⁵⁰.

A good starting point is to consider andesite genesis and to extend the argument later for granites. High pressure experimental studies⁵¹, ranging up to 40 kbar, indicate that primary andesite magmas can be produced by partial fusion of subducted, amphibolitised ocean crust^{51,52} corresponding to quartz eclogite¹⁵ after dehydration. However, certain other difficulties have been encountered in generating andesites by simply melting subducted ocean crust^{53,54} since 25–50% partial melting would be required to account for both the SiO₂ content and Fe/Mg ratios of average andesites. This degree of melting would dilute incompatible, large ion lithophile (LIL) trace elements yielding concentrations several times lower than observed even assuming their complete partitioning into the melt phase. Consequently, it has been necessary to devise rather more sophisticated mechanisms to account for andesite geochemistry and basically there are two possibilities: (1) Magma generation occurs within the subduction zone, satisfying major element andesite requirements, followed by enrichment in LIL elements during ascent by scavenging⁵⁶ of the overlying refractory ultrabasic mantle and, possibly, lower crust (2) The hydrous subducted lithosphere dehydrates^{51,57} releasing volatiles, already enriched in LIL elements, into the overlying dry ultrabasic mantle causing incipient melting there. Only 5–10% melting is required in this case to produce andesites which are more likely to satisfy both major and minor element constraints⁵⁴.

Following Ringwood¹⁵, Thorpe *et al.*⁵⁵ have provided an interesting bridge between these two ideas by deriving a garnet pyroxenite LIL-rich wedge over the subduction zone which is later able to melt giving andesite. This is a continuous process active so long as subduction continues. In this way, the variable initial ⁸⁷Sr/⁸⁶Sr ratios for andesites are produced⁴⁷ as radiogenic strontium accumulates for different times in a temporarily Rb-rich source region. However, Spooner⁵² has shown that some ocean crustal rocks may increase their ⁸⁷Sr/⁸⁶Sr ratios by hydrothermal exchange with seawater already enriched in a continent-derived strontium component. Involvement of such materials in the production of melt beneath a Cordilleran margin provides a source for initial strontium isotope variability directly, without necessitating a complex mechanism.

It seems that a mechanism for producing andesite magmas at mantle depths is evolving, but what of granite magmas? At first sight there are problems because partial fusion of upper mantle materials cannot directly produce acid liquids^{13,51}. However, if one defines the term “granite” more rigorously it is immediately obvious that the average composition of most batholiths lies between quartz diorite (tonalite) and granodiorite, rather than granite in the strict sense. Indeed, if we ignore the limitations of rock nomenclature based on grain size, the similarity between the material in batholiths and volcanic andesites on geochemical grounds is quite striking. W. S. Pitcher (personal com-

munication) and Myers⁵⁸ have commented on these geochemical similarities in Peru: increasingly it is becoming evident that many of the intrusive centres are fossilised magma conduits which fed the overlying extrusives. Apparently tonalite (that is, andesite) magmas rose by stoping and cauldron subsidence towards the surface, locally generating a varied compositional suite by the late stage secondary differentiation process described earlier. Some assimilation or partial melting of crustal material en route to the surface is probable, and is supported by the secular changes towards higher initial strontium isotope ratios during mid-Cretaceous to Quaternary Andean plutonic activity²¹. Indeed, this process may be an essential part of producing a refractory, Rb-poor basement. Crustal melting experiments are relevant here, and show that mantle-derived tonalite melts should enter the crust with enough thermal energy gradually to drain the lower crust of near minimum melting components. I would attribute the primary composition trend (see introduction) on a time scale of 10⁸ years in Cordilleran batholiths to the gradual raising of crustal temperatures during the active cycle so that both mantle magmas can assimilate at higher crustal levels to become relatively granitic, and these more granitic magmas can reach higher levels before being trapped by their melting curves^{12,59}. Of course, crustal assimilation is limited by the constraints discussed earlier in this paper, but an andesitic magma need only increase its volume by between 25 and 50% to resemble the most acidic, volumetrically significant adamellites of Peru. As more data become available further small changes towards systematically higher initial ⁸⁷Sr/⁸⁶Sr ratios (see ref. 21) should be traced through the primary time-composition trend of Cordilleran granites.

The consanguinity of intrusive and extrusive magmas at destructive plate margins seems to remove the experimental objections to furnishing batholiths at the expense of mantle. The arguments given in this paper suggest that those who advocate exclusive derivation of Cordilleran granites by reprocessing of existing continental crust should seriously contemplate a revision of their thoughts. The onus is on them to show how Andean-type crustal thickening and low initial ⁸⁷Sr/⁸⁶Sr ratios in Cordilleran granites are produced without mantle involvement.

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Rates, sample sizes, and the neutrality hypothesis for electrophoresis in evolutionary studies

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It is shown that electrophoretic genetic distance estimates are highly correlated with albumin immunological distances between the same pairs of species. The bimodality of rates of electrophoretic differentiation at various loci is then documented and the electrophoretic clocks involved are calibrated. The differences between the systematics and population genetics uses of electrophoretic data are then discussed. Finally, a direct electrophoretic test of the neutral alleles hypothesis is presented and exemplified on a set of Thomomys bottae-umbrinus data.

GENOMIC differentiation between diverging lineages begins as soon as their gene pools have developed a sufficient degree of reproductive isolation. Recently it has become possible to provide direct measures of the degree of such differentiation through the comparative biochemical techniques of amino acid sequencing, DNA hybridisation, electrophoresis, and immunology. As amino acid sequencing is much too time-consuming for routine application to evolutionary studies, and DNA hybridisation untested in detail, most of our current estimates of biochemical genetic distances derive from electrophoretic and immunological comparisons of proteins from species of interest. Electrophoresis, in particular, has become the favourite tool of

those wishing to apply protein data to problems in systematics and population biology. Nonetheless, few, if any, studies have been carried out to develop an appropriate molecular context within which the meaning of the electrophoretic data could be properly comprehended. We especially lack direct information as to the rates of amino acid substitution in the various proteins used in electrophoretic studies, which makes it difficult to tell whether the calculation of a single genetic distance from data involving numerous loci is a legitimate exercise, and, if so, how to relate these distances to a possible time scale. Our approach to this problem began with a comparison of electrophoretic and immunological distances for the same pairs of species. The results of that study led to an appreciation of the striking bimodality in the observed rates of protein electrophoretic evolution, of the ready availability and use of the set of rapidly evolving proteins to easily deal with levels of relationship heretofore inaccessible to the molecular systematist, and, finally, to the development of a direct test of the neutral alleles hypothesis in explaining electrophoretic polymorphisms.

We can begin with a definition of the distance measures used. For electrophoretic comparisons, Nei has introduced a measure of genetic distance, D , where D is the average number of electrophoretically detectable substitutions per locus between the two populations being compared^{1,2}. If x and y are the frequencies of the i allele in populations x and y , then the measure of genetic identity (I) is given by the equation

$$I_{xy} = \frac{\sum x_i y_i}{(\sum x_i^2 \sum y_i^2)^{1/2}}$$

where the summation is over all alleles and all loci compared, and $D = -\log I$.

For the immunological comparisons, we use the microcomplement fixation immunological distance (ID)³, where the ID between two homologous proteins is equal to five times the % sequence difference between them⁴. For the proteins whose immunology will be discussed here, albumin and transferrin, one ID unit will then be equivalent to about one amino acid substitution.

Electrophoretic genetic distance and time

The albumin immunological, and Nei electrophoretic, distances for 76 pairs of species were measured and compared (Fig. 1). There is obviously a strong correlation ($r = 0.82$) between the two measures of genetic distance, which, since different loci are being studied, suggests that each is a function of a third variable—most probably time. Ample documentation of the time-dependent nature of the vast majority of amino acid substitutions in a variety of organisms has been made in the last 15 years^{10,11}—so that, for example, one can speak of an albumin 'clock', and document that it generally keeps rather good time¹². Any such clock is necessarily a statistical one, and the comparison of two of them—in this case, the albumin and electrophoretic clocks—has to be carried with this fact well in mind. Each point in Fig. 1, considered in a time dimension, then becomes a probability ellipse. Because the number of substitutions counted in albumin immunological comparisons is appreciably greater than the number counted for the corresponding typical electrophoretic study involving 20–25 loci, the long axis of the ellipse will be parallel to the D axis. This is approximated in Fig. 1, where the dashed lines encompass an area within one electrophoretic standard error¹ of the regression line. The standard error is given by

$$\text{s.e.} = \left(\frac{1-I}{nI} \right)^{1/2}$$

where n is number of loci. To a first approximation, then, if the albumin and electrophoretic clocks were keeping perfect time, 2/3 of all the points should fall within the dashed lines. Remark-

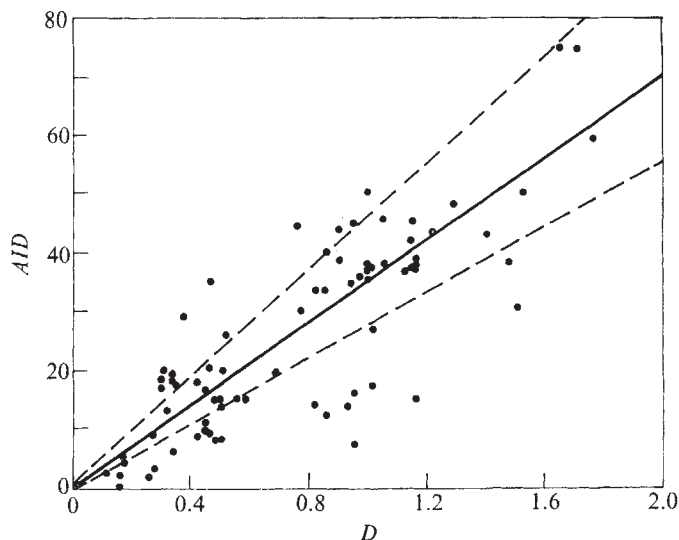


Fig. 1 Microcomplement fixation immunological distances are plotted against the electrophoretic Nei D values for the same species pairs. A preliminary version and discussion of these data have been presented previously (V.M.S., L. R. Maxson and A. C. Wilson, paper presented to the Society for the Study of Evolution, December 1973). The comparisons use published electrophoretic data on *Dipodomys*⁵, *Peromyscus*^{6,7}, *Hyla*⁸, and *Anolis*⁹. Other electrophoretic data on *Sigmodon* were provided by W. E. Johnson and R. K. Selander, on *Anolis* by G. C. Gorman, and on *Thomomys* by S. Y. Yang and J. L. Patton. All of the albumin immunological comparisons were carried out in this laboratory. The dashed lines indicate the standard errors of the electrophoretic D values. $r = 0.82$ (76 points).

ably enough, except for the cluster of seven points involving the kangaroo rat species *Dipodomys ordii*, they do. For *D. ordii*, presumably, relative to the actual phyletic distance separating it from other kangaroo rats, the albumin distance is uncharacteristically low or the electrophoretic distance too high. Immunological studies of *Dipodomys* and other geomyoid transferins⁷ give a distance between *ordii* and other *Dipodomys* species in line with the electrophoretic distance, suggesting that in this case it is the albumin immunological distance which is misleading.

It is thus evident that there is an electrophoretic clock and that it can be calibrated in terms of the albumin clock—not so much, it must be emphasised, because of earlier demonstrations of the strongly time-dependent nature of albumin evolution in other taxa, but because the rate of albumin evolution is known from those studies. Albumins accumulate some 100 units of immunological distance for every 60 Myr of separation¹², and, from Fig. 1, $AID = 35D$. Thus it takes, on the average, 20 Myr for a typical locus to accept an electrophoretically detectable substitution in one or the other of a pair of diverging lineages. Assuming a mean size of 400 amino acids¹³, and that 20% of all substitutions are electrophoretically detectable, this means that the average protein sampled in the usual electrophoretic studies has a unit evolutionary period¹⁰ in the area of 15–20 Myr. This very large figure is characteristic of the classic slowly evolving proteins such as cytochrome *c* and glyceraldehyde 3-phosphate dehydrogenase, and this makes one wonder as to the representativeness of these loci—especially when it comes to calculating times of divergence from electrophoretic data. For example, Nei's original calibration¹, which has been used in numerous divergence time calculations (see Table 2), set a D of 1 at about 3 Myr of separation—a figure almost an order of magnitude too low. An important complicating factor, however, has to be dealt with before realistic time of divergence calculations can be made.

Locus-dependent rates of change

This problem was first evidenced in the findings of King and Wilson for the *Homo-Pan* electrophoretic comparison^{14,15}. There the D value of 0.29 for the various 'intracellular' loci sampled stood in sharp contrast to the value of 2 for the plasma protein

Table 1 Plasma protein *D* values and albumin plus transferrin immunological distances between the same pairs of taxa

Taxa compared	Albumin plus transferrin distance	Plasma protein <i>D</i>
<i>Cercopithecus patas</i> – <i>C. aethiops</i>	3	0.4
<i>Papio</i> – <i>Theropithecus</i>	7	0.6
<i>Pan paniscus</i> – <i>P. troglodytes</i>	7	0.7
<i>Macaca nigra</i> – <i>M. fascicularis</i>	9	0.7
<i>C. patas</i> – <i>C. talapoin</i>	14	1.5
<i>Pan</i> – <i>Gorilla</i> – <i>Homo</i>	15	1.6
<i>M. nigra</i> – <i>Papio</i>	22	2.0
<i>Cercocebus albigena</i> – <i>C. torquatus</i>	26	2.0

loci. That this result is not a statistical fluke is documented in Table 1. We list there several plasma protein *D* values and the corresponding albumin plus transferrin immunological distances. As transferrin accumulates amino acid substitutions some 50–60% more rapidly than albumin^{16,17}, one thereby obtains immunological distances sufficiently large to be reliably compared to the plasma protein *D* values. For the data of Table 1, the summed immunological distance corresponding to a *D* value of 1 is about 10 units, of which about 4, on the average, will have been contributed by the albumins. Thus the *Homo*–*Pan* result, if anything, slightly underestimated reality, and the plasma proteins are accumulating amino acid substitutions some 10 time more rapidly than the enzymes sampled in the electrophoretic surveys which provide the bulk of the data of Fig. 1. Nor do we see evidence to date of any particular conservation in the rate of electrophoretic change in any particular plasma protein (for example, the plasmas of *Homo*, *Pan*, and *Gorilla* show only one band with the same mobility in all three); thus we are forced to the conclusion that a rather striking bimodality describes the distribution of electrophoretically measured rates of evolution at the various protein loci. In other words, there are two sets of proteins, and one of them is accumulating electrophoretically detectable substitutions at a rate tenfold greater than the other. This rapidly evolving set includes, in addition to the plasma proteins, various other 'secreted' proteins such as those found in saliva, egg-whites, and haemolymph, the non-specific esterases, and certain enzymes not involved in complex metabolic pathways—such as ribonuclease, lysozyme, and carbonic anhydrase; that is, many of the class 2 and 3 loci^{18–20}.

Setting the electrophoretic clock

This finding, as we shall see, has certain very important corollaries, not the least of which is the proper setting of the electrophoretic clock. As noted above, numerous authors have recently attempted to date divergence events among populations using electrophoretic data. These data typically, however, involve comparisons among both slowly and rapidly evolving proteins—with the latter usually including albumin, transferrin, haemoglobin, and one or more esterases. Clearly the findings reported here require a partitioning of the electrophoretic data set into two, as the clocks involved are obviously moving at grossly different rates. In essence, then, early in the process of genetic differentiation between two diverging lineages the bulk of any measured distance will be contributed by the rapidly evolving loci. This contribution will be essentially complete by 5–6 Myr of separation (that is, all the rapidly evolving proteins will have different electrophoretic mobilities by that time), and any

further accumulation of genetic distance will be seen only in the slowly evolving loci. Future studies then ought to focus on a large set of rapidly evolving loci for temporally close relationships, and on a comparably sized set of slowly evolving loci once one gets beyond 4–5 Myr of separation. Specific divergence times can then be calculated once the equation $AID \approx 35D$ is corrected for the fact that the data from which it was derived stem from studies averaging about 3/4 slowly evolving loci and 1/4 rapidly evolving. At a *D* of 1, $I = 0.37$, and effectively all this similarity will have been contributed by the slowly evolving loci—thus their actual *I* value will be $0.37/0.75$, or 0.5 ; corresponding to a *D* of 0.7. That is, in 35 Myr the set of slowly evolving loci will give a *D* value of 0.7, so that the corrected equations will approximate: (1) slowly evolving loci: $AID = 50D$; $T(\text{Myr}) = 30D$ (2) rapidly evolving loci: $AID = 4D$; $T(\text{Myr}) = 2.4D$.

We include, for heuristic purposes, a plot relating time of separation to *D* for a typical study where 3/4 of the loci are slowly evolving, and 1/4 rapidly evolving, as Fig. 2. In addition, we present in Table 2 some adjusted times of divergence involving data already in the literature and using the calibrations derived in this article. It might be noted that some of those adjustments produce substantial change which will have to be taken into account when reassessments of the evolutionary pictures implied are made.

Systematics against population genetics—sample sizes

At this point, before getting into more theoretical matters, it is appropriate to consider the distinction between population genetics and systematics uses of electrophoretic data. The basic interests of the population geneticist of course lie in the realm of population dynamics, and the knowledge of allele frequencies is an essential prerequisite to further work. The population geneticist, then, studies populations. The systematist, on the other hand, has as a first goal the knowledge of the evolutionary relationships among the organisms with which he is working. Thus, in assessing, for example, the evolutionary genetic distance between humans and chimpanzees, one human and one chimpanzee can give an entirely representative picture. The question therefore arises as to how many individuals per population will be necessary for a particular study, and clearly the answer to this question depends on the genetic distances involved, as well as on the rates of evolution of the loci being sampled. It then becomes vital to achieve some reliable assessment of the nature of the evolutionary relationships involved in the study at hand before, for example, undertaking the onerous and time-consuming task of surveying numerous populations with many individuals per population at 20 or more loci—as has all too typically been the case in much recent electrophoretic work. Such an assessment has to take place in stages, and it might be instructive to illustrate those stages with specific examples.

The first stage question would simply ask whether effective genetic isolation has been achieved between the populations in question. As the genetic differentiation which accompanies the development of such isolation will most readily be seen here with the set of rapidly evolving loci, one should first compare the various easily available secreted proteins—for vertebrates, this would mean the plasma proteins and the nonspecific esterases.

Table 2 Some published times of divergence calculated from electrophoretic data and adjusted according to the calibrations introduced in this article

Taxa involved and specific divergence event	Proportion fast loci	<i>I</i>	Published divergence time (Myr)	Calculated divergence time (Myr)
Icteridae (blackbirds)				
Agelaiine–Quiscaline groups	0.53	0.7	0.27	1.4 ref. 21
<i>Thomomys talpoides</i> (gophers)				
48-chromosome New Mexico populations and others	0.36	0.845	0.23	1.2 ref. 22
<i>Peromyscus</i> (deer mice)				
<i>boyleyi</i> and <i>truei</i> species groups (excepting <i>polius</i>)	0.47	0.68	0.24	2.1 ref. 23
<i>Drosophila</i>				
<i>virilis</i> – <i>littoralis</i> – <i>montana</i> species groups	ca0.7	0.37	0.75	3.9 ref. 1

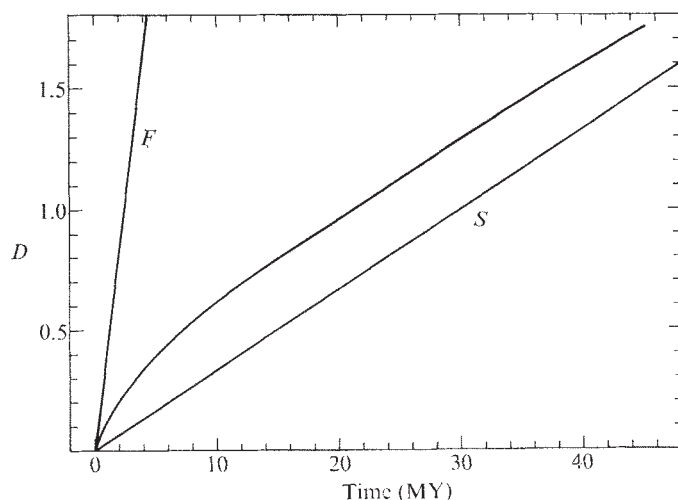


Fig. 2 The accumulation of electrophoretic genetic distance (D) as a function of time for the rapidly evolving proteins (F), slowly evolving proteins (S), and a mixed system involving 3/4 slowly evolving, and 1/4 rapidly evolving proteins (unlabelled line) calculated according to the calibrations discussed in the text.

With reasonably fresh material one ought to be able to readily see some 25–30 bands on a single gel. These could be compared most simply in two individuals as the number with the same mobility divided by the number counted in both. Thus the only way that two individuals drawn from the same gene pool could give a I value of less than 1 would be for them to be homozygous for different alleles at one or more loci. The maximum probability for such an occurrence is $1/8$ (at a polymorphic locus with two alleles at a frequency of 0.5)—thus even if $1/3$ of our 25–30 loci were polymorphic, it would be unlikely for the two individuals to be counted as different at more than one locus. Even this rather low ‘noise’ level can be effectively eliminated by comparing two individuals per population—the $1/8$ probability per locus then drops to $1/128$. Beyond this one gains nothing until a large enough number of alleles is counted so that one can reliably speak of allele frequencies, and the required 30 or so individuals now increases the workload by more than an order of magnitude without anything approaching a commensurate gain in effective resolving power. In fact, if one takes into account observed heterozygosity values and the evolutionary rates calculated here, it becomes evident that very little indeed in the way of resolving power is contributed to the systematics of a group by that component of genetic distance due to allele frequency differences at the slowly evolving loci. Resolving power over the last few million years of relationship is far more efficiently gained through the use of the rapidly evolving set of loci—most simply by the band counting approach just outlined, and ultimately by allele frequency comparisons at those loci which are polymorphic. Appropriate attention to these considerations, with emphasis on increasing the number of slowly evolving loci in the study, should then greatly facilitate routinely working with evolutionary relationships extending over the last 50 MY or so ($D_{\text{slow}} = 1.6$). If, for example, one could routinely work with 40 slowly evolving loci, this would mean that the effective rate of electrophoretic substitution along any given lineage is one per 1.5 Myr; that is, the average ultimate resolving power is 1.5 Myr up to about 50 Myr of separation. It is, of course, 10–50-fold better in the range 0–6 Myr due to the ready availability of a large set of rapidly evolving loci.

A practical test of this ‘band-counting’ approach to the electrophoretic systematics of closely related forms is available among the kangaroo rats (*Dipodomys*). Patton, MacArthur, and Yang have recently reported²⁴ that *Dipodomys heermanni* is clearly composed of two quite distinct species, with one more closely related to a third, *D. panamintinus*, than it is to the other *heermanni* (now renamed *californicus*). That study surveyed 22 loci and some 100 individual kangaroo rats. Yet one gel with one

individual per population at issue—using band-counting for the plasma proteins and liver esterases—can give congruent results; although, of course, the D values obtained are appreciably higher (Table 3). It should also be noted that for many taxa of interest populations are not realistically available, and this approach enables one to learn a great deal about relationships at taxonomic levels heretofore mysterious using sample sizes of only one or two individuals per population. Such a preliminary effort among the Old World monkeys, complementing an already completed immunological study, has already been reported²⁵. We have also been able to obtain the first reliable measures of genetic distance separating the pygmy chimpanzee (*Pan paniscus*) from the more common *Pan troglodytes*, and the Eastern and Western lowland gorillas. In each case the plasma protein D value was 0.7, suggesting separations on the order of 2 Myr ago. The corresponding *Pan-Homo-Gorilla* distances are 1.6–1.7, consistent with the summed albumin and transferrin immunological distances among the three of 15 units (Table 1).

These results also suggest that some degree of caution should be exercised in the use of genetic distance criteria to assess the taxonomic levels at which populations ought to be separated. Obviously the genetic distances obtained will be very dependent on the loci included in the study, and a ‘species level’ I value of 0.85 for a study involving a preponderance of slowly evolving loci might well become a ‘genus level’ value of 0.3 when looking at the rapidly evolving set. Thus the $D. heermanni$ – $D. panamintinus$ I value of 0.9 (ref. 24), which might be used to argue for conspecificity, becomes 0.6 when the plasma proteins and liver esterases are used. One can, however, foresee a possible negative effect of this newly increased resolving power when dealing with lineages of relatively recent origin. This will stem from the marked proliferation of recognisably different (at the genetic level, at least) populations which are not exchanging genes (that is, which have fixed different alleles), and which, therefore, can be seen as representing different ‘species’. For mammals, this proliferation will certainly occur among the rodents—and one can only hope that this increased resolving power might be used to raise our level of understanding of the dynamics of the evolutionary process, and not to make it so much easier to play the ‘splitting’ game in formal taxonomy.

A direct test of the neutral alleles hypothesis

Finally, we can consider the test of the neutral allele hypothesis. We know that, for a neutral allele polymorphism, the length of time a given lineage remains polymorphic will depend only on the size of the populations along it. The probability of a given locus being polymorphic, on the other hand, is a function of the rate of amino acid substitution at that locus. Thus rapidly substituting loci will be polymorphic more often, thereby being more likely to contribute to population heterozygosity—again, to emphasise, for neutral allele situations. For such situations, then, heterozygosity per locus will be proportional to the rate of amino acid substitution at that locus. To the extent that this is actually descriptive of a body of data, we argue that such a data set is consistent with the neutral hypothesis; to the extent that it is not, we are encouraged to look for specific selective effects. One case where the neutral hypothesis does seem entirely adequate to explain the observed data involves a study of 25 *Thomomys bottae-umbrinus* (pocket gopher) populations, where at least 30 individuals per population were surveyed at 23 loci

Table 3 A comparison of the results obtained with the plasma proteins and liver esterases using one individual per population with those obtained in the usual population genetics approach in *Dipodomys*

	D.h.(5)	D.p.	D.h.(4)
<i>Dipodomys heermanni</i> (5-toed)	0	0.47	1.60
<i>Dipodomys panamintinus</i>	0.08	0	1.35
<i>Dipodomys heermanni</i> (4-toed)	0.24	0.15	0

The numbers above the diagonal are the plasma protein plus liver esterase D values using one individual per taxon. Those below the diagonal were obtained using 18 ‘slow’ and 4 ‘fast’ loci with a total of 100 individuals²⁴.

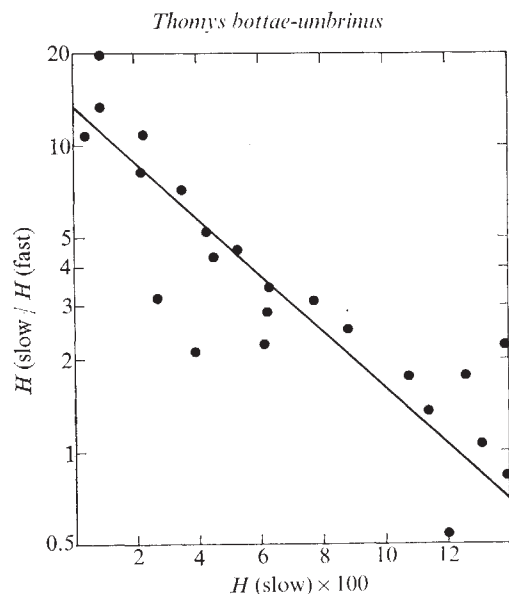


Fig. 3 The ratio of heterozygosity (H) values in a series of *Thomomys bottae* and *umbrinus* populations for sets of 18 slowly evolving, and 5 rapidly evolving loci are plotted against the H values for the slowly evolving set. The justification for the log-linear plot is presented in the text.

(18 'slow', 5 'fast')²⁵. The H values for the 'fast' and 'slow' sets of loci were then calculated, and plotted as $\log H(\text{fast})/H(\text{slow})$ against $H(\text{slow})$ for each population (Fig. 3). The log transform is to allow for the fact that the number of potentially detectable electrophoretic alleles in a population is limited by the integral nature of charge changes—thus the $H(\text{fast})/H(\text{slow})$ ratio could not increase as a linear function of $H(\text{slow})$. It should be noted that $H(\text{fast})$ clearly does increase as $H(\text{slow})$ increases and, extrapolating to $H(\text{slow}) = 0$ gives us a $H(\text{fast})/H(\text{slow})$ intercept of 13, which is, considering the statistical uncertainties involved, remarkably similar to the $D(\text{fast})/D(\text{slow})$ value of about 12 derived previously. It is difficult to see how this quantitative congruence between rates of substitution and heterozygosity levels at particular sets of loci could be the result of anything other than the random walking of neutral alleles.

Summary and suggestions for further work

These findings should be considered indicative of an area in which a great deal of further work needs to be done, and not necessarily representative of a general pattern. The data of Fig. 1 certainly argue forcibly for the strongly time-dependent nature of rates of electrophoretic differentiation, but there is, nonetheless, adequate space left for a certain degree of selective main-

tenance of various polymorphisms. Appropriate further testing of the neutral allele hypothesis in the manner just described awaits a sufficiently large sampling of rapidly evolving loci so that the sample sizes of the two sets of loci are comparable. This should be a relatively simple matter in most vertebrate groups, where one should certainly be able to routinely score 15 plasma protein, and 5–10 esterase, loci. For the very large and important *Drosophila* data set, however, with which so much of the neutralist-selectionist controversy has been concerned, it will be necessary to document a reasonable sample of rapidly evolving loci prior to carrying out these tests. Presumably these would be most readily available in the haemolymph and among the larval proteins and non-specific esterases, though clearly the rates of differentiation at the various enzymatic loci ought also to be compared to see if any of those are especially rapidly evolving. The available data, then, are explicable within a simple framework involving a very small number of variables, and the next step consists of expanding the taxonomic base within which this simple framework can be seen as operative.

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T4 Bacteriophage-coded RNA polymerase subunit blocks host transcription and unfolds the host chromosome

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T4 bacteriophage mutants selected for their ability to grow with cytosine in their DNA are defective in host transcriptional shutoff and host chromosome unfolding. The host RNA polymerase purified from cells infected by such a mutant lacks a small T4-coded subunit.

T4 BACTERIOPHAGE shuts off host transcription efficiently and rapidly but the molecular mechanism behind this remains a mystery. One reason for the mystery may be that no T4 mutants defective in host shutoff have yet been isolated. Since T4 probably uses the host RNA polymerase for all its transcription^{1,2}, inactivation of the polymerase cannot be involved in

shutoff. During infection however, various T4 coded polypeptides bind to the host RNA polymerase. These include the products of T4 genes 55 (refs 3 and 4) and 33 (refs 3 and 5) and of two other unidentified genes. The latter two polypeptides, molecular weights 15,000 and 10,000, were assigned the numbers 2 and 4, respectively, by Stevens³. There are other polypeptides which bind loosely to the RNA polymerase⁶. In addition, the α subunit of the RNA polymerase is adenylated⁷ by two distinct T4-coded activities, *alt* and *mod* (ref. 25), one of which, *alt*, is carried in the virus particle^{8,9}. The adenylated RNA polymerase transcribes *E. coli* genes poorly *in vitro*²⁹. Neither *alt* nor *mod* mutants affect the shutoff of host protein or rRNA synthesis *in vivo*⁸, however, and none of the T4-coded RNA polymerase subunits has been implicated in host shutoff.

Besides changing the host RNA polymerase, T4 infection alters the host "chromosome". The host DNA changes its intracellular location³¹ (nuclear disruption), a process requiring the T4 *ndd* gene product¹⁰. The highly compact structure of the host nucleoid is unfolded¹², requiring the product of the T4 *unf* gene¹¹. The host DNA is degraded to mononucleotides chiefly by the products of genes *denA* *endoII* (refs 13 and 14) and 46, 47 (ref. 15). All of these phenomena can proceed independently of each other^{10,11}. None of them has been related in a simple way to the shutoff of the synthesis of at least the major host proteins¹¹.

T4 has hydroxymethylcytosine in its DNA instead of the usual cytosine¹⁶. It is tempting to speculate that the shutoff of host transcription and/or replication occurs when T4 causes the cell to reject DNA with cytosine for some aspect of transcription and/or replication. If this is true, T4 with cytosine in its DNA might "shut itself off". T4 mutations which permit the multiplication of cytosine-containing T4 would occur in genes normally involved in host shutoff. To test this hypothesis, and to find T4 regulatory genes, we have begun the search for T4 mutants which can produce T4 with cytosine in their DNA.

The first such mutants we have found are deficient in the function of a gene which we call *alc* (ref. 17). When cytosine-containing T4 DNA is made after infection, the *alc* gene product almost completely prevents true-late transcription. True-late mRNAs are those which require T4 DNA replication¹⁸ to have occurred and the products of genes 33, 55 (ref. 18) and 45 (ref. 19) to be present. Here we present evidence that the product of the *alc* gene becomes an RNA polymerase subunit, probably the polypeptide no. 2 of Stevens³. It is also required to unfold the host nucleoid after infection and is likely to be the *unf* gene product of Snustad and his collaborators¹¹. We discuss the implications of this for the structure of the host nucleoid, and the relationship of the structure to transcriptional regulation.

Missing T4-coded polypeptide in *alc* RNA polymerase

In the original report on *alc*⁻ mutants, we proposed that the *alc* gene product, itself non-essential, interacts with an essential part of the transcription apparatus. We proposed that this may be one of the activities which adenylate the α subunit of the RNA polymerase or may be one of the T4-coded RNA polymerase subunits. Since mutants are available which lack one or the other adenylating activity⁸, we tested these first. Neither *alt*⁻ nor *mod*⁻ mutants were able to confer the *alc*⁻ phenotype after recombination with T4 which makes cytosine-containing DNA. Thus, we tentatively concluded that *alc*⁻ mutants are not simply *alt*⁻ or *mod*⁻. We then purified the RNA polymerase from cells after infection by an *alc*⁻ mutant to test for the presence of the T4-coded polypeptides. Figures 1a and b show polyacrylamide gels of RNA polymerase from *alc*⁺ and *alc1* (*alc*⁻) infected cells respectively. The *alc*⁻ mutant is conspicuously lacking the major peak of radioactivity in about fraction 33, but has all the others. By molecular weight determinations with known markers and by comparison with the published results of Stevens³, we think that the missing peak is the 15,000 molecular weight polypeptide no. 2. *Alc1* was a spontaneous mutant of the *alc*⁺ parent shown in Fig. 1a. Thus, we are quite sure that the *alc*⁻ mutation caused the absence of the polypeptide in the RNA polymerase. In

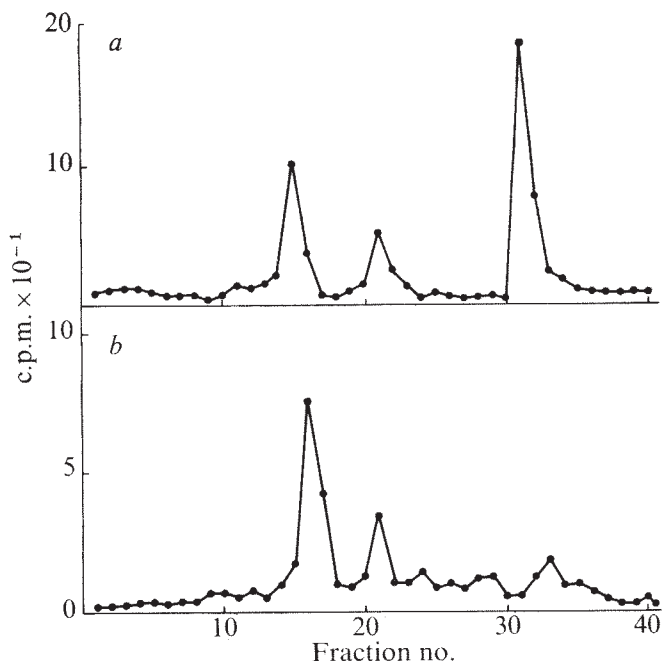


Fig. 1 An *alc*⁻ mutant is lacking a T4-coded polypeptide on the RNA polymerase. *E. coli* B⁺ (100 ml) growing in M9 without amino-acids at 30 °C were labelled with ³H-leucine 5–15 min after infection. They were then mixed with 1 g of uninfected cells, sonicated, and the RNA polymerase purified by the method of Burgess²¹ through phosphocellulose chromatography. The results of slicing and counting 12% SDS gels (procedure of Fairbanks *et al.*²²) is shown. The dye front was on the right. a, T4-coded polypeptides on the RNA polymerase from the parent of the spontaneous *alc*⁻ (*alc1*) mutant (b). The *alc*⁻ mutant is clearly missing the first peak from the right in about fraction 34. We think this peak is due to Stevens' polypeptide no. 2.

addition, we have done this experiment using 'mixed labels'. The *alc1* mutant infected cells were labelled with ³⁵S-methionine and the *alc*⁺ parent with ³H leucine. The infected cells were then mixed and the RNA polymerase purified as before. The polypeptide in question was only labelled with ³H offering additional evidence that, after infection by the *alc1* mutant, the polypeptide is not bound to the RNA polymerase. This is not true for all apparent *alc*⁻ mutants, however. We shall return to this point later. The *alc1* mutation does not seem to affect the synthesis of any other T4 early gene products. Thus, *alc* is probably the structural gene for this RNA polymerase-bound polypeptide.

Map position of *alc*⁻ mutations

The *alc* gene product seems to be relatively non-essential in most laboratory conditions. We have found, however, that the multiplication of some *alc*⁻ mutants, including *alc1*, is temperature sensitive. This effect is amplified in certain RNA polymerase mutants of *E. coli* of the Rif^R-2 type²⁴ and *alc1* will not form plaques at 42 °C on these strains. We think the temperature sensitivity is due to the *alc*⁻ mutation because (1) a fairly high percentage (~10%) of spontaneous *alc*⁻ mutants are temperature sensitive, and (2) we have not been able to separate the temperature sensitive and *alc*⁻ phenotypes by recombination.

Even though we do not know the molecular basis of the temperature sensitivity we have used it to map *alc*⁻ mutations. First, we isolated the *alc1* mutation by crossing out the amber mutation and the rII deletion which were used in its selection¹⁷. We then crossed this temperature-sensitive mutant with amber mutants in genes around the T4 map and selected for non-temperature sensitive, *am*⁺ recombinants. We obtained the lowest recombination frequencies with mutants in the region of gene 31. To localise the mutation more precisely, we constructed double mutants of the temperature-sensitive mutant and amber mutants in genes in this region. We then crossed these against

Table 1 Map position of *alc*⁻ mutations

Cross Crossover occurring	<i>am</i> H21, 54	<i>alc</i> ⁻	× <i>am</i> H39 <i>alc</i>	<i>am</i> H39, 30	<i>alc</i> ⁻	× <i>am</i> H54 <i>alc</i>	<i>am</i> N54, 31	<i>alc</i> ⁻	× <i>am</i> M69	<i>am</i> M69, 63	<i>alc</i> ⁻	× <i>am</i> N54 63
	30			31			63			31		
Expected frequency		~1			~1			~1			~1	
Observed frequency		50			57			43			56	
		56			65			112			97	
Map order: 54	30	31	<i>alc</i> 63									

Double mutants *am*, *alc*⁻ were constructed by crossing and screening *am* progeny for temperature sensitivity by spotting on K803 Rif^r-2²³ at 42 °C. These double mutants were then crossed with single amber mutants in the region of gene 31. The *am*⁺ recombinants were spotted on K803 Rif^r-2 to determine their temperature sensitivity. Shown is the fraction of the total *am*⁺ progeny from one plate which are *alc*⁻. The amber mutants used were *am* H21 (gene 54), *am* N54 (gene 31) and *am* M69 (gene 63). The *alc*⁻ mutant is *alc*1, the same mutant used in the RNA polymerase experiment in Fig. 1. *alc*1 is also *unf*⁻. We have also analysed other crosses, the results of most of which were also consistent with this map order.

single amber mutants and tested the *am*⁺ progeny for their temperature sensitivity (Table 1). We have shown the map order which is consistent with the data and the crossover occurring in each case. The data support the map order gene 63-*alc*-gene 31.

There are four other types of mutations which have been reported to map between genes 63 and 31. These are *rIII*, *cd*, *pset* and *unf* (ref. 11). Of these, *unf* may be related to *alc* since they both might be expected to play a part in host shutoff, *unf* because it is required for the unfolding of the bacterial nucleoid and *alc* because it prevents a type of transcription on cytosine-containing T4 DNA. Thus, we studied the ability of *alc*⁻ mutants to unfold the host nucleoid after infection.

alc⁻ Mutants defective in host nucleoid unfolding

In 1971, Stonington and Pettijohn reported a lysis procedure which leaves *E. coli* DNA highly folded, perhaps in a similar state to its intracellular structure²⁰. These compact structures, nucleoids, have a high sedimentation rate and contribute little to the viscosity of lysates. If they are unfolded, however, after T4 infection¹² for example, their S value drops markedly and the lysates are extremely viscous.

To see whether *alc*⁻ mutations affect the T4-induced unfolding of the host nucleoid, we made sucrose gradients of nucleoids from uninfected, *alc*⁻ infected and *alc*⁺ infected cells (Fig. 2). In agreement with others, our nucleoids from uninfected cells sedimented heterogeneously with an S value of about 2,000. This S value was largely unchanged by infection with the *alc*⁻ mutant while it dropped to about 1,000S after infection by *alc*⁺ T4. Thus, the *alc*⁻ mutant is defective in host nucleoid unfolding.

We also determined the time course of unfolding by *alc*⁻ mutants by measuring the viscosity of lysates of cells taken at different times after infection. The results for two independent *alc*⁻ mutants and their parent are shown in Fig. 3. Both *alc*⁻ mutants were defective in host nucleoid unfolding compared with the parent. One of them, *alc*2, probably never unfolds the host nucleoid whereas the other, *alc*4, is merely delayed about 1–2 min in unfolding. Some *alc*⁻ mutations do not measurably affect the timing or extent of host nucleoid unfolding.

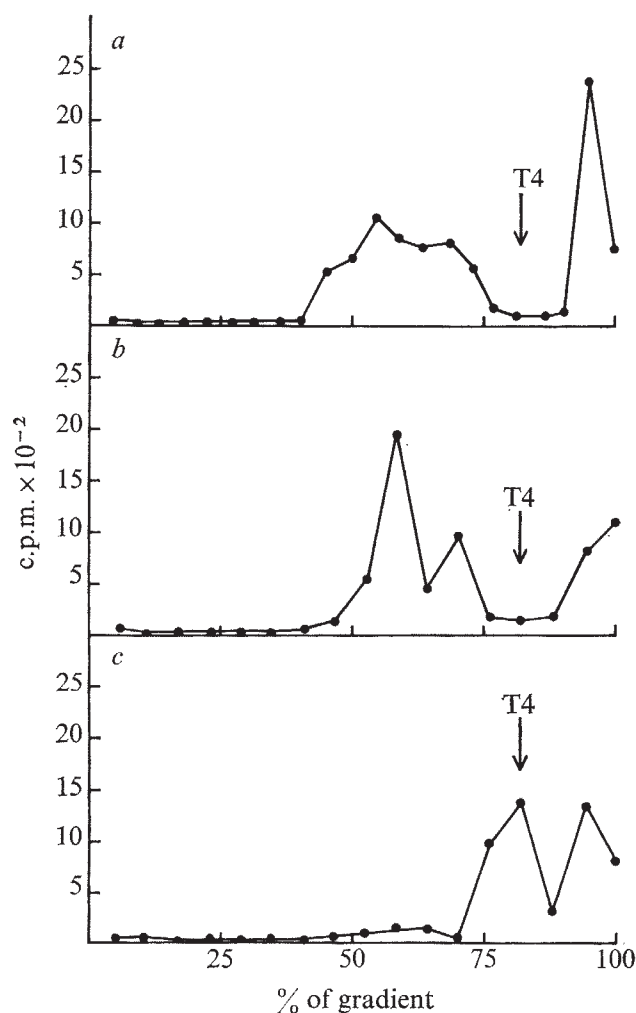


Fig. 2 Sucrose gradients of nucleoids after infection by an *alc*⁻ mutant. *E. coli* B^E (5 ml) growing at 30 °C in M9S were labelled for 1 h before infection with 16 μ Ci ml⁻¹ thymidine (5.5 mCi mg⁻¹). They were infected at a concentration of 4×10^8 ml⁻¹ at a multiplicity of infection of 5 in 10 μ g ml⁻¹ tryptophan. At 5 min after infection, 1.0 ml was poured into a chilled tube containing 0.1 ml of 1 mg ml⁻¹ chloramphenicol. The lysis procedure of Snustad *et al.*¹¹ was used except that the solutions were made up as follows. Solution I: 0.1 M Tris, 6.5 mg sodium azide, 1.0 g sucrose, 0.58 g NaCl, and 9 ml H₂O. Solution II: 1.2 ml Tris, 2.0 ml EDTA, 4 mg lysozyme, and 6.8 ml H₂O. Solution III: 0.1 ml Sarkosyl, 1.16 g NaCl, 0.5 ml EDTA, and 9.0 ml H₂O. Tris was 1 M adjusted to pH 8.15 with HCl. EDTA was 0.25 M adjusted to pH 7.0 with NaOH. After 5 min for lysis, two additional steps were performed before layering on gradients. 0.1 ml of 20.0 mg ml⁻¹ lysozyme dissolved in solution II were added for 10 min and then the lysate was diluted with 2.0 more ml of solution III. Five minutes later the lysate was chilled and 0.15 ml were layered on 4.7 ml 10–30% w/v sucrose gradients made up with 0.5 ml Tris, 0.25 ml EDTA, 0.25 ml Sarkosyl, 5.8 g NaCl, and 10 μ l mercaptoethanol per 50 ml. There was a 0.5-ml step at the bottom made from a solution containing 0.1 ml Tris, 6.0 g sucrose, 1.0 g NaCl, 3.0 ml saturated CsCl and 3.0 ml H₂O. They were centrifuged at 4 °C for 25 min at 17,000 r.p.m. in a Spinco SW50L rotor. Fractions were collected from the bottom which is at the left. a, Uninfected; b, *am*E51, DD2, *alc*2 (ref. 17), an *alc*⁻ mutant induced by hydroxylamine and back crossed three times against its parent at a ratio of 1 : 10; and c, the *alc*⁺ parent of *alc*2. The arrows show the peak of sedimentation of a T4 phage marker.

Are *alc* and *unf* the same gene?

Several considerations support the conclusion that *alc* and *unf* code for the same gene product. A fairly high percentage (> 10%) of *alc*⁻ mutants are also measurably *unf*⁻. Those *alc*⁻ mutants which are not measurably *unf*⁻ are also those which make reduced amounts of late gene products when cytosine-containing T4 DNA is made (data not shown). We have also obtained what seem to be partial revertants of the *alc1* mutation (that is, they are less *alc*⁻). These have also become *unf*⁺ to varying degrees. The temperature-sensitive mutation which we have mapped is inseparable from *alc* by recombination and maps in the same region as the *unf* mutation of Snustad *et al.*¹¹. So far, those *alc*⁻ mutations which confer the most temperature sensitivity have been the most completely *unf*⁻. The *alc2* mutant used was back-crossed against its parent three times at a ratio of 1:10, selecting the *alc*⁻ phenotype each time; it was still *unf*⁻. All of these observations support the conclusion that *alc* and *unf* are the same gene. However, we have not rigorously excluded two other possibilities. One of these is that *alc* and *unf* are neighbouring genes and those *alc*⁻ mutants which are also *unf*⁻ are deletions spanning these two genes. The existence of partially *unf*⁻ mutants such as *alc4*, however, which is also intermediate in its temperature sensitivity and *alc*⁻ phenotype, argues against the deletion hypothesis. Also, the high frequency of *alc*⁻, *unf*⁻ mutants has already been given as evidence for point mutants. The other possibility is that *alc*⁻, *unf*⁻ mutants are double point mutants, *alc* and *unf* are adjacent genes, both the *alc*⁻ and *unf*⁻ mutations enhance the multiplication of cytosine-containing T4, and the temperature sensitivity is due to the *unf*⁻ mutation. However, we think these alternative explanations are very unlikely and that *alc* and *unf* are the same gene.

If *alc* and *unf* are the same gene why are not all *alc*⁻ mutations measurably *unf* deficient? The most likely explanation is that T4 mutants which are only partially defective in *alc* function can still multiply with cytosine in their DNA to make a plaque; but are not as temperature sensitive and are scored as *unf*⁺ in our unfolding test.

Implications for host shutoff and *E. coli* nucleoid structure

Although *alc* might be expected to play a part in host shutoff, *alc*⁻ and *unf*⁻ mutants do not affect the timing of shutoff of synthesis of the host proteins one sees on polyacrylamide gels (ref. 11 and our unpublished observations). Since there could be a translational shutoff superimposed on the transcriptional one, however, we looked at the shutoff of host RNA synthesis directly by testing to see whether any RNA synthesised after infection by an *alc*⁻ mutant can hybridise to *E. coli* DNA. The results are shown in Table 2. It is clear that the *alc*⁻ mutation

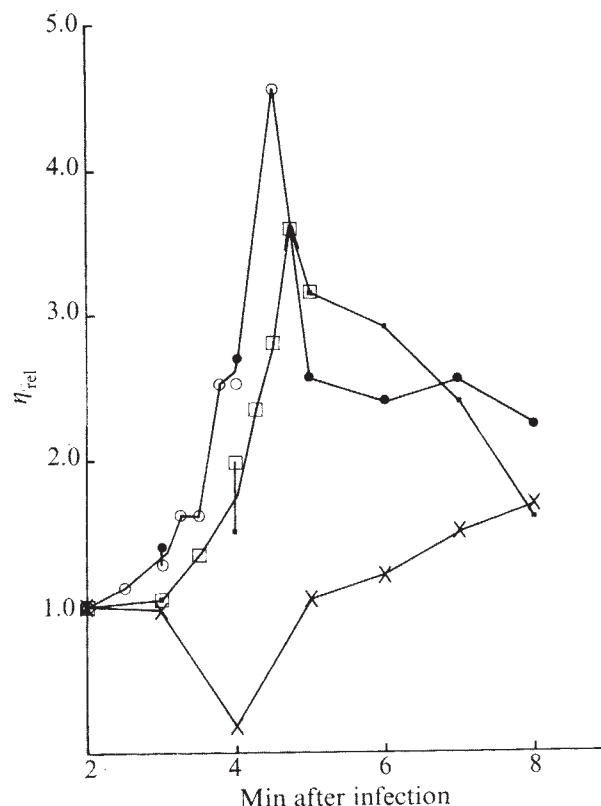


Fig. 3 Time course of unfolding of host nucleoids after infection by *alc*⁻ mutants. Cells were infected and all 5 ml lysed as in Fig. 2. 12 min after solution III was added, the viscosity of the lysate was measured without previous centrifugation by measuring the rate of movement in mg s⁻¹ through a 20-gauge needle 4 cm long. The ratio of this number to that obtained from lysates of cells at 2 min after infection is plotted. Lysates of uninfected cells flowed at close to the same rate as cells collected 2 min after infection and both were only slightly slower than H₂O. The process of measuring the viscosity of all the samples took less than 15 min and the uninfected cell lysates were still not viscous at this time. The closed and open figures of each type represent different experiments. We drew the line through the midpoint when the results were slightly different for the same time after infection. (○), *amE51*, *DD2* (ref. 17); (□), *amE51*, *DD2*, *alc4*; (×), *amE51*, *DD2*, *alc2*.

affects the shutoff of the synthesis of at least some *E. coli* transcripts. Thus, selecting T4 mutants which could multiply with cytosine in their DNA has led to the discovery of a host shutoff gene, *alc*.

Table 2 Hybridisation of RNA labelled after infection by an *alc*⁻ mutant to *E. coli* DNA

RNA ³ H	Labelling time	c.p.m. Input	c.p.m. Hybridised	% of RNA Hybridised	% of RNA which is <i>E. coli</i> (100)
Uninfected T4 ⁺	2-min pulse	8,397	1061	12.7	17.8
	4-6	4,736	107	2.26	8.2
	8-10	3,033	31	1.04	31.5
	14-16	2,557	103	4.0	34.6
<i>alc1</i>	4-6	5,048	224	4.4	43.0
	8-10	3,495	196	5.5	72.5
	14-16	2,840	261	9.2	

RNA was pulse labelled at the times indicated in M9 medium at 30 °C with 10 μCi ml⁻¹ ³H-uridine (5 Ci mmol⁻¹). The phage were *alc1* which had been crossed against the wild type (T4⁺) to remove the *amE51* and *DD2* mutations¹⁷. The multiplicity of infection was 10 and tryptophan was added at 10 μg ml⁻¹ before infection. Surviving bacteria were less than 0.1% at 2 min after infection. The RNA was extracted three times with phenol at 60 °C by the Method I of Bolle *et al.*¹⁸, ethanol precipitated, treated with DNase (5 μg ml⁻¹) in 0.1 M Tris-Cl pH 8.0, 0.01 M MgCl₂ for 15 min at 37 °C, extracted twice and reprecipitated with ethanol. Hybridisation was with 20 μg ml⁻¹ *E. coli* DNA³⁰ for 40 h at 60 °C in 2SSC. The hybrids were treated with RNase (12 μg ml⁻¹ RNase A at 37 °C for 15 min), collected on presoaked Millipore filters, and washed with 0.5 M KCl, 0.01 M Tris, pH 7.5. The specific activities of the RNAs were from top down, 10.6, 6.76, 4.0, 3.32, 7.8, 5.85, and 4.27 c.p.m. ng. The percentage of RNA which is *E. coli* was determined by dividing the percentage of input counts which hybridised by the percentage of the uninfected labelled RNA which hybridised. This number is affected by changes in the relative concentrations of *E. coli* RNA species so should only be considered qualitative. It is perhaps surprising that *E. coli* transcription can continue so late into infection when the host DNA is being degraded. This RNA is presumably being made on fragmented DNA.

If *alc*⁻ mutants are defective in the shutoff of host transcription, why are they normal in the shutoff of the host proteins one sees on gels? One possibility is that there is a translational shutoff which is independent of transcriptional shutoff, and evidence has been presented for such a translational shutoff by T4 (refs 27 and 28).

Host RNA is still being made after infection even by *alc*⁺ phage, as also observed by Kennel²⁷. In fact, at late times the percentage of RNA synthesis attributable to the host apparently increases (see Table 2). Either *alc* is inefficient in its shutoff of host transcription or it is only responsible for shutting off a subset of the host RNA synthesis. Accordingly, we are undertaking experiments to determine whether all types of host transcription are shut off equally by *alc*.

In conclusion, we have presented evidence that an RNA polymerase subunit can cause the host nucleoid to unfold. Since mutants which lack the function of this subunit allow increased transcription of the host genome after infection, perhaps the *alc* gene product prevents the synthesis of RNA which holds the nucleoid together. In this connection, it has been reported that treating cells with rifampin causes the unfolding of the nucleoid²⁶. Other types of experiment also suggest that RNA is important in maintaining the highly folded state of the host nucleoid since RNase treatment can cause it to unfold²⁰. According to one model, then, the unfolding of the host chromosome is merely a consequence of shutting off host transcription by *alc*. However, another possibility should be considered: the unfolding of the host chromosome by *alc* may cause host transcription to cease. If so, perhaps T4 does not make RNA on cytosine-containing T4 DNA because *alc* causes it to "unfold itself". It should be possible to design experiments to test these, and other, hypotheses. Because of this, the T4 *alc* gene product promises to be an important tool in the study of the modes and mechanism of prokaryotic transcrip-

tion. It also promises to be useful in the study of the roles of RNA and RNA polymerase in maintaining the structure of the prokaryotic chromosome.

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letters to nature

Evidence from optical polarisation for a galactic-scale uniform magnetic field in M104

LINEAR polarisation of light has been observed in several galaxies¹⁻⁴, but the only previous detailed polarisation pattern has been mapped in M82^{2,3,5,6} from which information about the luminous and physical structure of the galaxy has been deduced⁷. Clearly, detailed knowledge of the polarisation pattern in a nebula is a powerful tool for further understanding of the nebula. In this letter we present a map of the linear optical polarisation of the Sombrero Galaxy (M104) giving the first clear evidence for the existence of a magnetic field which maintains the same projected direction over an estimated volume of some 10³ kpc³.

The observations were made on the 1-m telescope of the Wise Observatory, Israel during the period May 20-24, 1976 using the B waveband of the UBV system. The technique used has already been described⁸ and uses the Durham polarimeter and the Royal Greenwich Observatory 4-cm electronographic camera to record the analysed light on nuclear emulsion. The electronographic plates have been digitised on the PDS machine at the Royal Greenwich Observatory with a bin size of 1.4 × 1.4 arc s. The polarisation measurements have been determined at each point over an integration area of 7 × 7 arc s (that is, over 25 digitisation bins). Measurements

have been made at 1,000 points within the galaxy. Calibration checks on the performance of the polarimeter have been made using standard polarised stars as references⁸. It has not been necessary to make instrumental corrections.

The resulting polarisation map is shown in Fig. 1. Our technique eliminates any non-uniformity of the electronographic emulsion as we make two independent measurements at each point and these agree in all cases on this map. Each measurement is indicated by a line in the direction of the E-vector, proportional in length to the degree of polarisation, and centred on the point observed. The map is superimposed on a photograph of M104. The polarisation in the dark lane has an average value of 2.2 ± 0.5% and position angle of 86° ± 5°. A previous single measurement in this area by Appenzeller⁴ is in agreement with our values if we integrate over a similar area to that used by Appenzeller.

The main features of the polarisation map are: (1) An almost uniform polarisation parallel to and along the central dark lane spanning an area at least ~ 100 × 30 arc s, which at a distance of 12 Mpc (ref. 9) is equivalent to 6 × 1.6 kpc. The orientation of the polarisation is also maintained over the brighter part of the lower optical lens. (2) In the upper lens the electric vectors are perpendicular to the dark lane. The transition from parallel to perpendicular orientation of the electric vectors is abrupt.

In Fig. 2 we show the intensity (*I*) and polarised intensity (*P*) as functions of position along a scan line perpendicular to the dark lane and through the nuclear region. We note several

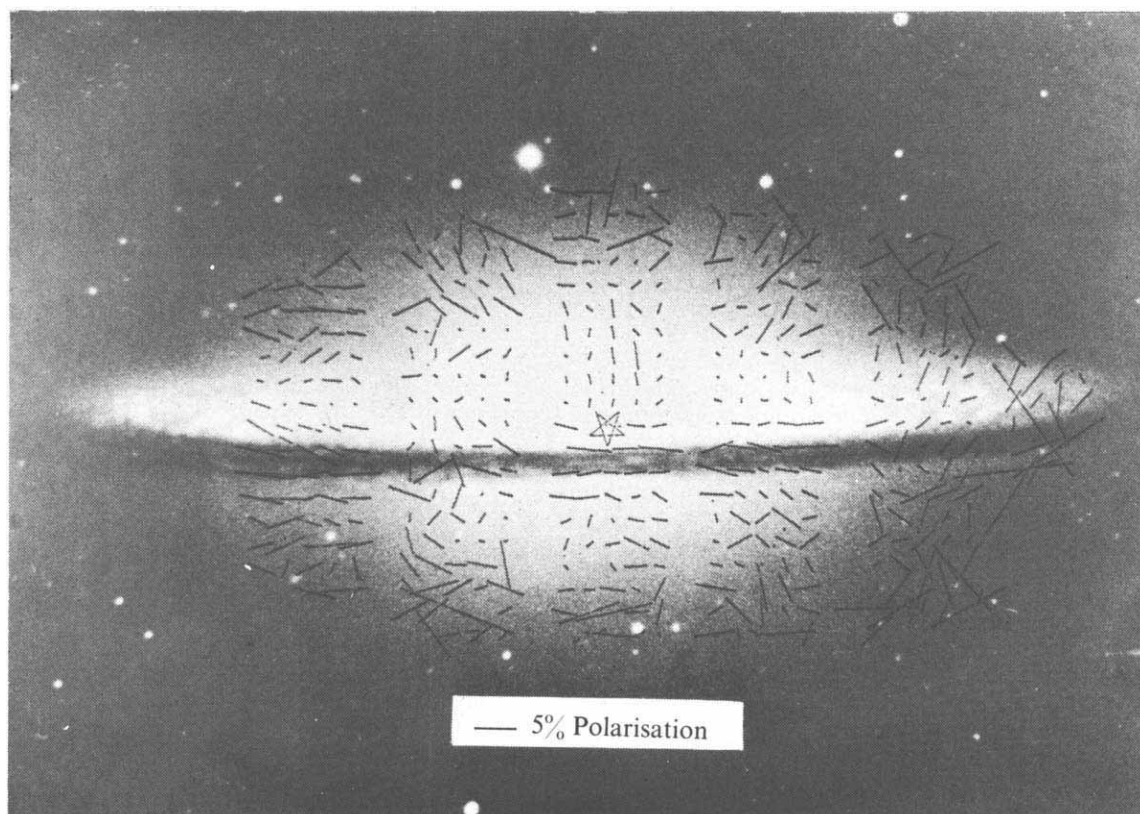


Fig. 1 The linear optical polarisation of M104 in the B waveband. *Represents position of nucleus.

features of the graphs. (1) The centre of the dark lane is readily apparent in the graph of I ; it is at the pronounced dip and below the maximum of I . (2) The maxima of I and P are not coincident; the former maximises ($I_{\max}$) at 21 arc s N of the dark lane and the latter ($P_{\max}$) at 14 arc s N. (3) While neither P nor I is symmetrical about its maximum, P is more symmetrical. The change from parallel to perpendicular alignment of the E-vectors occurs between the maxima of P and I .

Finally we have integrated over the whole electronographic plate and estimate that the apparent nucleus contributes approximately 20% to the total luminosity.

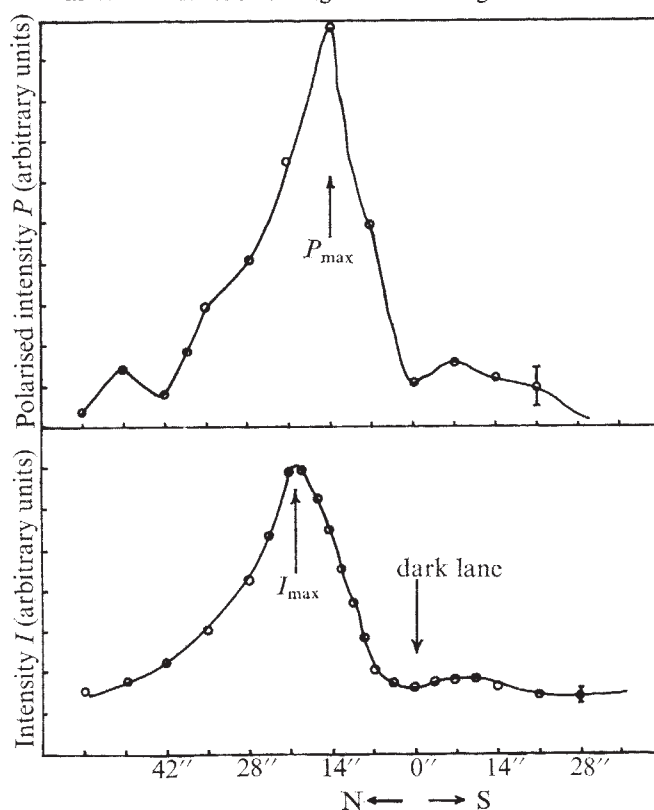
There is little doubt that the observed polarisation pattern is due to the scattering and extinction of light by statistically aligned dust grains. Synchrotron emission is eliminated as M104 is neither a radio¹⁰ nor an X-ray source¹¹. Rayleigh scattering can also be eliminated as the illumination of an optically thin Rayleigh atmosphere by a nucleus plus disk produces a distinctive centro-symmetrical pattern⁷ quite unlike that observed.

The alignment of the dust grains is most probably due to magnetic effects as proposed by Davis and Greenstein¹². The alignment in our Galaxy has been the subject of much discussion; radiative transfer of angular momentum by optical¹³ and X-ray¹⁴ photons and gas streaming¹⁵ are alternative processes to the Davis-Greenstein effect for dust alignment. However, it is difficult to see how the alternatives could be effective in the case of M104 as the optical photons would produce alignment in the wrong sense to that observed; for gas streaming the alignment would be slowly changing with no polarisation observed at the outer edge of the disk: this again is not observed. Thus unless some heretofore unexpected mechanism is at work the grains must be magnetically aligned.

The change in orientation of the polarisation vectors from perpendicular to parallel to the dark lane is probably the result of increasing dust density towards the plane of the galaxy and a corresponding change of polarisation by scattering to extinction. This then implies that the magnetic field maintains its orientation not only for another 2 kpc above the galactic plane but in depth as well.

The condition for the transition in orientation is that the polarised intensity from scattering (P_s) of disk light must exceed the polarised intensity from extinction (P_e) of halo starlight beyond 21 arc s above the dark lane. Since light scattered by aligned dust grains¹⁶ (submicrometre prolate spheroids) is polarised by some 50% (for scattering optical

Fig. 2 Graphs of the intensity and polarised intensity for a scan in the N-S direction through the central region of M104.



depth $\tau_s < 1$) we have $P_s \approx 0.5 \tau_s L_D / 4\pi h^2$ where L_D is the galactic disk luminosity (nucleus included) and h is the height above the disk. P_e is given by $P_e \approx 3.10^{-2} e(-\tau_e) n_* L_* Z / 4\pi$ where n_* and L_* are the density and luminosity of halo stars, Z is the line of sight distance through the halo, and τ_e is the optical depth for extinction. A polarisation of 3% is assumed for extinction. For $P_s > P_e$, one has

$$\tau_s L_D > 0.1(1 - \tau_e) L_H$$

where $L_H \approx n_* h^2 Z L_*$ is the total luminosity of halo stars in the volume $h \times h \times Z$. Since $L_D > L_H$, this condition is easily satisfied far above the disk.

As h decreases however, the densities of both dust and stars increase, and stars which were illuminating dust above the galaxy begin to contribute to L_H , with a corresponding decrease in L_D , so that extinction begins to dominate. For low values of τ_s , small changes in n_* and $\tau_e \approx \tau_s$ can change the character of the polarised light completely, and this transition is what one observes 21 arc s N of the dark lane's centre. Evidently, the condition is not satisfied in the southern hemisphere of the galaxy, and extinction predominates.

From the fact that the $I_{\max}$ and $P_{\max}$ are not coincident, we infer that the brightest parts of the nucleus and disk are obscured. In fact, it seems that the (obscured) brightest part of the galaxy is between 7 arc s and 14 arc s N of the dark lane centre, near where $P_{\max}$ occurs. Here the optical depth τ_e must be larger than one, so that over some 14 arc s, τ_e varies by about two or three orders of magnitude. This implies a scale height of 100–200 pc for the dust, which is a typical galactic scale.

The fact that scattering from aligned grains is occurring makes M104 a likely candidate for observation of circular polarisation. If recent calculations¹⁶ are applicable, then a circular polarisation of some 15% is introduced in the scattered light, which means we expect some 0.7% circular polarisation to be observable after dilution by halo starlight. The maximum degree of circular polarisation should be in the halo, but since the optical depth is large in the dark lane, the maximum absolute amount may be observed there. An observation of circular polarisation would tend to confirm our interpretation of the linear polarisation.

We have presented here our preliminary results, and fuller analysis and detailed models will soon follow. It seems, however, that we are seeing the most extensive homogeneous magnetic field observed so far and it is tempting to speculate that it may be primordial in origin.

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Verification by Viking Landers of earlier radio occultation measurements of surface atmospheric pressure on Mars

UNTIL this year, the most accurate means of studying the atmospheric pressure at the surface of Mars were provided by remote sensing from fly-by or orbiting spacecraft. Radio occultation, which involves observations on Earth of the effects of refraction on the radio link from the spacecraft in the Martian atmosphere, has been used repeatedly to measure the pressures and temperatures in the atmosphere of Mars from the US Mariner¹ and Soviet Mars² spacecraft. By far the largest number of these measurements, some 260, was obtained with the Mariner 9 orbiter in 1972–73³.

The landing of Viking 1 in Chryse Planitia on July 20, 1976 provided the first opportunity to obtain measurements of atmospheric pressure directly from the surface of Mars. In the interest of comparing and verifying radio occultation results relative to the Viking *in situ* measurements, a computation was carried out to predict the atmospheric pressure at the landing site before the landing itself.

Because no radio occultation measurements existed near the Viking 1 site (22.3°N, 48.0°W), it was necessary to have a means of obtaining the relative altitude between occultation points and the Viking 1 site. This link was provided by Earth-based planetary radar data taken in 1967 (ref. 4), covering Martian latitudes from 19°N to 24°N. Four Mariner 9 occultation points lying within these latitudes were used to compute the distance from the centre of Mars (radius) of the zero-elevation reference level of the radar elevation profile (ref. 4, Fig. 4). These were points 77N (20.8°N, 337.7°W), 78N (24.1°N, 154.7°W), 418N (24.9°N, 180.9°W), and 420N (22.0°N, 172.4°W). The number designating the occultation measurement refers to the number of the revolution of the orbiter about Mars, and *N* stands for a measurement taken during entry into occultation. Combining the radii measured at those four points with the elevations indicated by the radar profile yielded a zero-altitude radius of $r_0 = 3389.54 \pm 1.5$ km. The uncertainty was estimated on the basis of uncertainties in both the radar and occultation measurements.

The predicted pressure at the Viking 1 site can then be estimated either with reference to a 6.1-mbar areoid computed from the gravity field of Mars and Mariner 9 occultation data⁵ or by direct comparison with occultation points consistent with the areoid. The results are displayed in Table 1.

The quantity Δh is the altitude difference between the Viking 1 landing site and the 6.1 mbar areoid in the first instance, and the radii measured at the respective occultation points in the second two. These are then used, assuming a 12-km scale height (corresponding to the expected atmospheric temperature at the time of Viking 1 landing) to extrapolate the pressures shown in the column labelled 'source' to the pressure at the Viking 1 site, which is given in Table 1, column 3.

These estimates of pressure are valid for the season of Mariner 9 measurements. Because of the interaction of the atmosphere of Mars with the polar caps, however, the global atmospheric pressure is expected to vary over a Martian year by as much as 1.7 mbar⁶. Although much uncertainty is attached to these estimates of seasonal variation, which will be definitely established by Viking measurements, it was estimated that the pressure should be lower by about 0.2 mbar at Viking 1 arrival relative to Mariner 9. This correction was made, and the corrected figures appear in Table 1, column 4. Finally, an average of the three determinations was taken, yielding a prediction of 7.6 ± 1.1 mbar.

The mean diurnal atmospheric pressure actually measured by Viking 1 was 7.65 mbar by the meteorology experiment⁷ and 7.3 mbar by the entry science experiment⁸.

The near exact correspondence of the measured and predicted pressure is of course fortuitous in view of the 1.1-mbar uncertainty attached to the prediction. Nevertheless, it indicates that the radio occultation results from Mariner 9 closely correspond to the actual surface pressure on Mars, which should enhance the credibility of the radio occultation method and of results obtained from planets and satellites whose atmospheres have not been directly probed.

Table 1 Estimates of pressure

Source	Altitude difference Δh (km)	Pressure at Viking 1 site (mbar)	
		Mariner 9 season	Viking 1 season
Areoid	-2.93 ± 1.5	7.8 ± 1.0	7.6 ± 1.0
$p = 6.1$			
Occ. pt 420N	-2.46 ± 1.5	7.5 ± 1.1	7.3 ± 1.1
$p = 6.7 \pm 0.5$			
Occ. pt 78N	-0.67 ± 1.5	8.1 ± 1.2	7.9 ± 1.2
$p = 7.65 \pm 0.5$			
Average, corrected for season			7.6 ± 1.1

A more cursory prediction was made for the Viking 2 landing site (48.0°N , 225.7°W). In the absence of planetary radar data, a Mars contour map compiled from Mariner 9 data⁹ was used to obtain altitude differences, and less reliable extended mission data were used. After correction for seasonal variations, a pressure of 6.7 ± 1.4 mbar was predicted. The actual pressure as measured by the meteorology experiment (unpublished data) was 7.72 mbar, within the uncertainty bounds of the prediction.

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Search for superheavy elements in monazite by Rutherford backscattering

EVIDENCE that superheavy elements with $Z=116$, 124 and 126 may exist in monazite inclusions associated with giant haloes in biotite mica has recently been reported¹. As there is no evident reason why such elements should not also exist elsewhere, it would seem fruitful to search for superheavy elements in monazite inclusions not associated with giant haloes, or even in bulk monazite, although their concentrations in such environments may be considerably lower than in giant halo inclusions.

In this letter, we report the results of such a search in samples of bulk monazite by a technique capable of detecting concentrations of order 1 p.p.m. A beam of ^{32}S ions is Rutherford scattered² backwards from the sample and the energies of the scattered ions are measured. The energetics of the scattering process are described by classical mechanics; at exactly 180° , for example, the energy of a particle of mass m and incident energy E_i scattered by a nucleus of mass M is just $E_{sc} = ((M-m)/(M+m))^2 E_i$ for $M > m$; if $M < m$ no scattered events will be observed and this is the case for all angles greater than 90° . As the target mass M increases, so also does the energy of the scattered particle. With the incident energy known, a measurement of the scattered energy can therefore be used to deduce the mass of the target nucleus.

We have used beams of 42 MeV ^{32}S ions from the Oxford Tandem van de Graaff accelerator to bombard targets of monazite sand obtained from the mineralogical collection of the University Museum and originating from India. The sand was finely powdered and glued on to aluminium backings; the resulting monazite targets were sufficiently thick (several mg cm^{-2}) to stop the beam. No backscattered events from the aluminium or from the constituents of the organic glue can occur because these elements are lighter than sulphur. The scattered sulphur ions were detected at a mean angle of 165° , the largest angle at which our detectors could be placed without interfering with the incident beam. Two thick silicon surface barrier detectors ($50\ \mu\text{m}$ and $75\ \mu\text{m}$) were used on opposite sides of the beam. The primary constituents of monazite, essentially (Ce, La, Y, Th) PO_4 , which can scatter sulphur ions into the backward hemisphere are the lanthanides (mainly Ce and La), Th and U. The phosphorus and oxygen are again lighter than sulphur and thus will not contribute. In the Table, we give the maximum energies of the backscattered sulphur ions from these and a possible superheavy constituent, under the experimental conditions mentioned above.

The energy spectrum of backscattered sulphur ions obtained from a 10 h exposure with one of the detectors is shown in Fig. 1. (A similar spectrum was obtained with the other detector.) Notice that the vertical scale is logarithmic, but with zero included; energy is plotted along the horizontal axis. The sharply cut off edges correspond to the maximum scattered energy from each of the elements indicated; i.e. from the target surface. The plateaux extending down to zero energy are due to the fact that the incident or scattered ion beam can lose all or part of its energy in the target.

If elements with $A > 238$ are present, then scattering from such nuclei would give rise to events above the Th+U edge (Table 1). Figure 1 shows the position of the edge corresponding to $A=354$ which is a predicted mass number for the most stable isotope of element 126 (ref. 3). However, such events can also arise from 'pile-up' caused by the simultaneous detection of two (or more) pulses of lower energy, and this limits the sensitivity of the technique. In

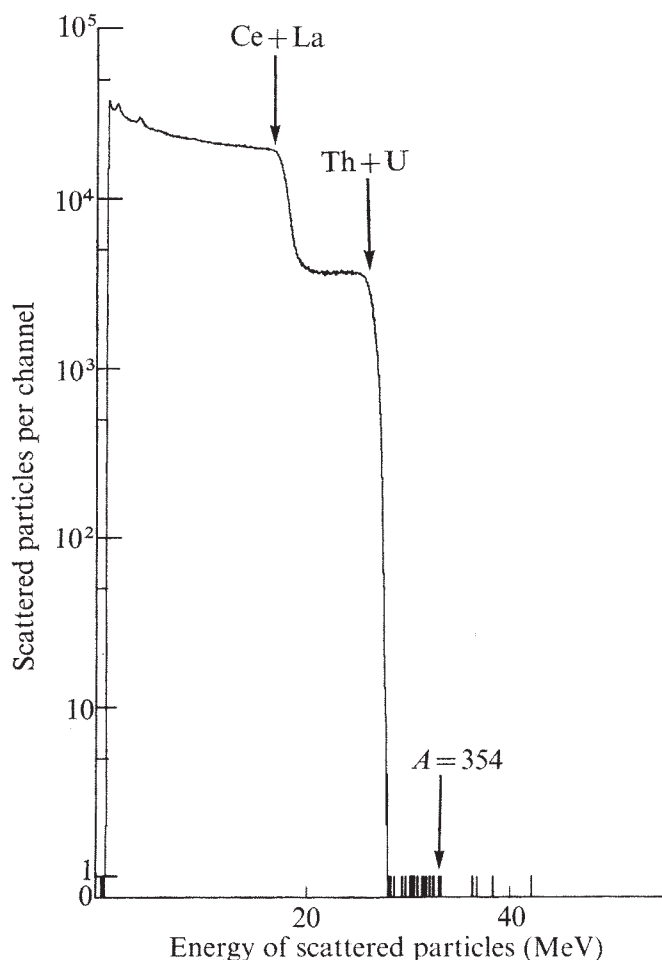


Fig. 1 Energy spectrum of ^{32}S ions of incident energy 42 MeV scattered from a target of powdered monazite sand observed at an angle of 165° . The edges correspond to scattering from the various constituents of the target (see text). An energy of ~ 24.5 MeV corresponds to channel 500 and there are an average of < 0.2 counts per channel in the region between the Th+U edge and the calculated position of the edge for $A = 354$.

the spectrum shown, there are 21 counts above the Th+U edge. Even under the improbable occurrence that these events were all due to scattering from superheavy nuclei, the level of the rare earths in Fig. 1 implies an upper limit of 2 atomic p.p.m. of superheavy nuclei of mass 354 (and approximate charge 126) relative to the concentration of the rare earths (mainly Ce and La) in our sample, where the Z^2 dependence of the Rutherford scattering cross section has been taken into account. (An analysis of the spectrum obtained with the other detector gave an upper limit of 1 atomic p.p.m.).

By using heavier projectiles than sulphur (for example, iodine or uranium) this limit might be reduced by a significant factor. A uranium beam is particularly attractive as only scattering from target nuclei with $A > 238$ can contribute to the spectrum of scattered particles observed at

Table 1 Calculated energy of ^{32}S ions, incident energy 42 MeV, with observation at 165° after scattering from various heavy constituents of monazite

Element	Mass number	Energy (MeV)
Ce	140	16.81
Th	232	24.33
U	238	24.67
(126)	354	29.40

A value is included for a possible superheavy element with mass 354.

angles greater than 90° . Such beams may well permit the detection of superheavy nuclei, if they exist, at very low concentrations indeed.

We thank Dr F. B. Atkins, Curator of the Mineralogical Collections of the University Museum for the provision of the monazite and Dr M. A. Grace and Dr H. J. Rose for valuable suggestions.

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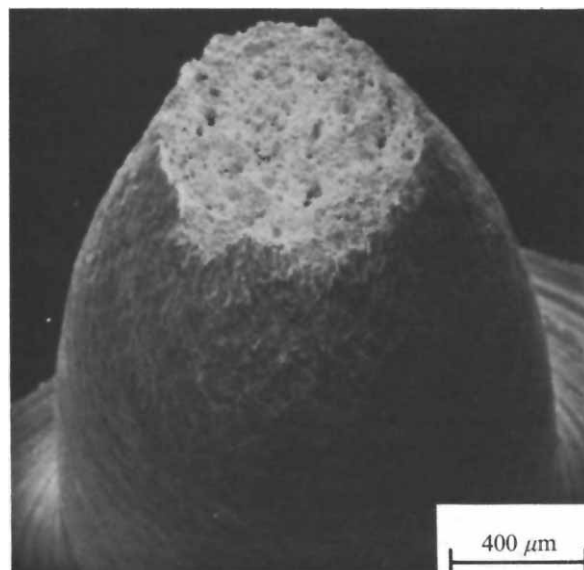
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Cadmium metal embrittlement of Zircaloy-2

EFFECTS of chemical environments upon the mechanical properties of zirconium alloys are of great interest because such alloys are used to contain fissionable fuel in water-cooled nuclear reactors. Particular interest attaches to stress corrosion cracking or liquid metal embrittlement because both of these phenomena are known to produce non-ductile fractures in otherwise ductile materials. Rosenbaum *et al.* reported iodine stress corrosion cracking of Zircaloy-2 some years ago^{1,2}, and this phenomenon has been extensively investigated by others³⁻⁵. Zircaloy-2 is an alloy of zirconium containing by weight about 1.5% tin, 0.15% iron, 0.1% chromium, 0.05% nickel and 0.12% oxygen. Cox has reported embrittlement of Zircaloy by mercury or liquid caesium at room temperature³. However,

Fig. 1 Scanning electron micrograph of a fracture surface of Zircaloy-2 broken in caesium at 300°C ; elongation rate 2×10^{-3} inch min^{-1} , sample gauge length 0.13 inch.



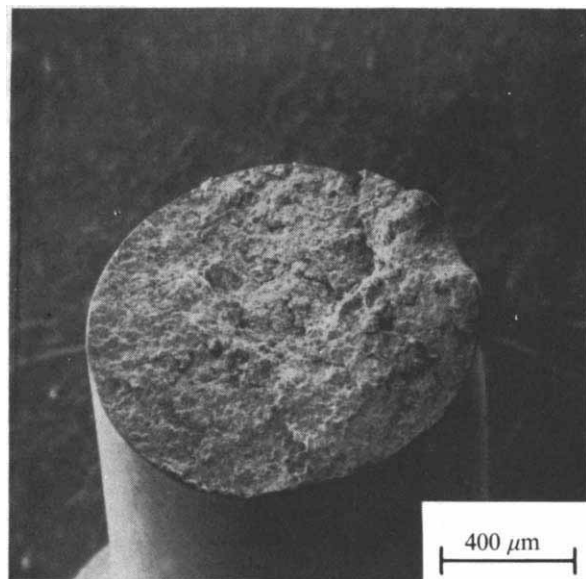


Fig. 2 Scanning electron micrograph of a fracture surface of Zircaloy-2 broken in caesium saturated with cadmium at 300 °C; other conditions as in Fig. 1.

the phenomenon of liquid metal embrittlement is known to be favoured by low temperatures⁶, and Zircaloy nuclear fuel cladding operates in the vicinity of 300 °C.

It is the purpose of this note to report the observation of embrittlement of Zircaloy-2 in the temperature region of 300–340 °C by cadmium. Embrittlement occurred in the presence of solid cadmium at 300 °C, liquid cadmium at

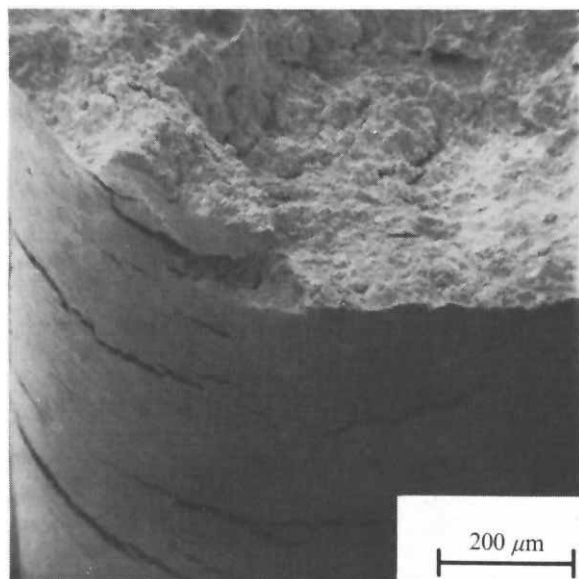


Fig. 3 A portion of the sample of Fig. 2 showing side cracks.

occurred with a reduction of area of about 75% and shows a typical ductile morphology similar to that observed in an inert argon environment. The fracture in caesium plus cadmium occurred with substantially zero reduction of area, and the fracture surface seems non-ductile. A portion of that surface at higher magnification is shown in Fig. 4. The mode of fracture is clearly transgranular with quasi-cleavage faces. It is, in fact, similar in appearance to fracture surfaces obtained by breaking Zircaloy-2 in iodine vapour at 300 °C (ref. 3).

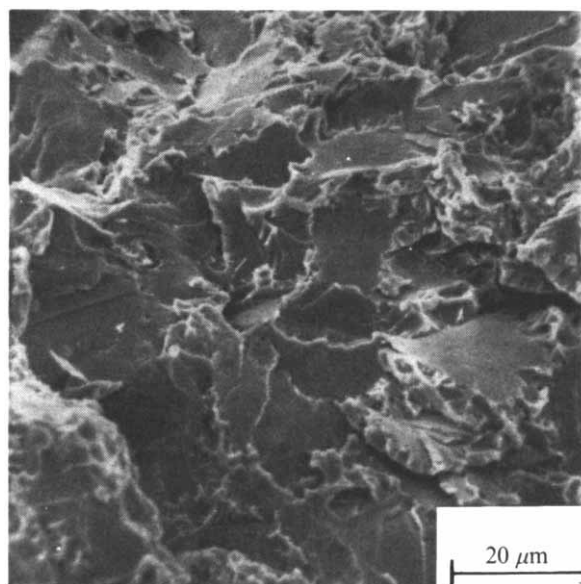


Fig. 4 A portion of the fracture surface of Fig. 2 at high magnification.

The tensile samples of Figs 1–4 were smooth-bar samples cut in the thickness direction from cross-rolled Zircaloy-2 plate. The plate was prepared according to a mechanical schedule described by D. Lee¹⁰ to provide a high degree of orientation of the *c* (0001) axis in the thickness direction. Cracks caused by cadmium seem to occur along or near the basal plane perpendicular to the *c* axis and the applied stress axis, as Figs 2 and 3 show.

These results seem to be the first observation of cadmium embrittlement of Zircaloy-2. I am investigating the phenomenon further; the experimental techniques and further results will be reported in the near future.

I thank Morris H. Morgan III for experimental assistance, D. Lee for samples and advice on cross-rolled Zircaloy, and A. W. Urquhart for discussions.

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340 °C, or cadmium dissolved in liquid caesium at either temperature. On the other hand, caesium alone did not embrittle Zircaloy-2 over this temperature range. Embrittlement of titanium alloys by solid or liquid cadmium has been reported by others^{7–9}.

Figures 1, 2, and 3 show fracture surfaces of samples broken in caesium (Fig. 1) or caesium saturated with cadmium (Figs 2, 3) at 300 ± 10 °C. The fracture in caesium

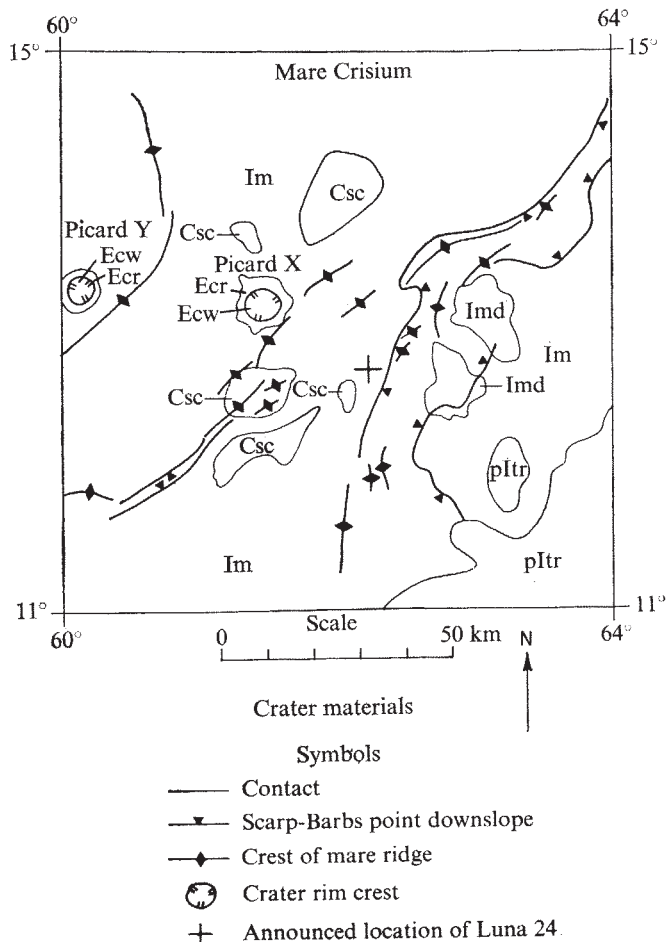
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Age of Luna 24 mare basalts based on crater studies

THE recent return of regolith material from Mare Crisium by Luna 24 has provided an opportunity to test our knowledge of lunar impact-flux history. This can be done by comparing crater density data for the landing site with a calibrated impact flux curve to estimate the absolute age of the sample before chemical analysis.

Luna 24 returned samples from south-eastern Mare Crisium (12.7°N, 62.2°SE). The area around the landing site was mapped (Fig. 1) in detail from Apollo panoramic photographs. Obvious swarms of secondaries and endogenic craters were excluded from the crater counts and only one unit was counted at a time. This was to ensure that only the crater (production) population was counted for a single unit. Two major mare units occur in the mapped area; Imbrian mare (Im) on which Luna 24 landed, and dark (low albedo) Imbrian mare (Imd). An additional mare unit may be present just north of the mapped area according to the data of Neukum, König, and Arkani-Hamed¹. Crater counts were made in two areas, one containing 2,920 km² (120 craters counted) of unit Im (including the landing site) and the other 256 km² (37 craters counted) of Imd, using Apollo panoramic and metric photographs. Diameter-cumulative frequency curves were constructed from these crater counts.

Fig. 1 Geological map of the Luna 24 landing site. Crater Materials: Csc, Copernican secondary craters; Ecw, Eratosthenian crater wall; Ecr, Eratosthenian crater rim (hummocky facies). Other materials: Im, Imbrian mare; Imd, Imbrian mare dark; pItr, pre-Imbrian terra rugged.



These curves are shown in Fig. 2. C_s (ref. 2), the intersection of Trask's³ steady-state curve and the extension of the approximately -3 slope crater production curve, is also shown in Fig. 2 for each of the three major mare units in and around the Luna 24 landing site and the six Apollo landing sites¹⁻⁷. The location of C_s values (which are a measure of impact flux) shows that the Luna 24 landing site is more densely cratered than the Apollo 12 and 15 sites, and less cratered than the Apollo 11, 17, 16 and 14 sites. C_s

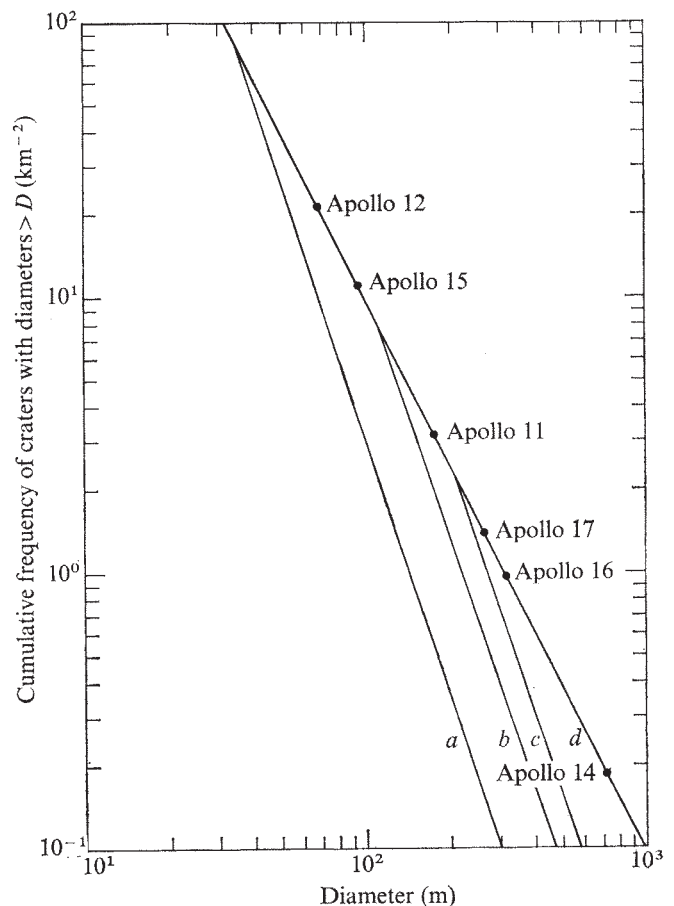


Fig. 2 Cumulative size frequency of craters on the three major mare units (Im, Imd, and the northern unit) in the vicinity of the Luna 24 landing site. The curves are extended to the steady-state curve of Trask's³ to obtain the C_s values for each curve. C_s values for each Apollo landing site are also included for comparison. a, Luna 24 northern unit; b, Luna 24 landing site (Im); c, Luna 24 (Imd); d, $N = 10^{1.9} D^{-2}$.

values for the Apollo landing sites can be compared with Apollo radiometric ages⁷⁻¹¹ to establish the lunar impact-flux history. This curve is shown in Fig. 3 and can be used to estimate the age of the Luna 24 site which is about 3.5 Gyr with a probable error of ± 0.1 Gyr. Since the returned sample is of regolithic materials it could also contain basalt fragments which have been transported into the area by impact from the other nearby units. Although this is unlikely, there may be basaltic fragments from the other two units included in the Luna 24 core that are about 3.75 ± 0.1 Gyr (Imd) and 2.5 ± 0.3 Gyr (northern unit).

Additionally, information about the geochemistry of the Luna 24 basalts can be determined from spectral reflectance measurements. Spectral reflectance data¹³⁻¹⁵ indicate that most of southern Mare Crisium (including the Luna 24 landing site) is 'red' low-titanium basalt similar to that at

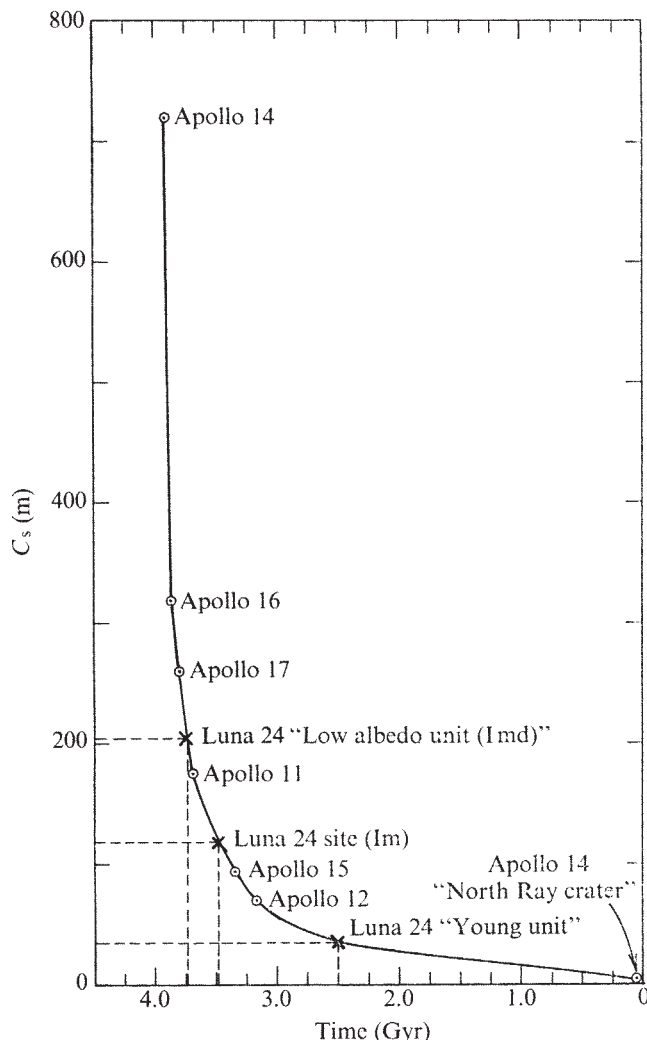


Fig. 3 Comparison of C_s values (a measure of impact flux and relative age) and crystallisation ages of Apollo samples. The estimated ages of the three units that may have been sampled by Luna 24 are included.

the Apollo 15 site. Therefore, based on crater frequency data and spectral reflectance data, we suggest that the mare basalts sampled by Luna 24 are similar in age and composition to those sampled by Apollo 15.

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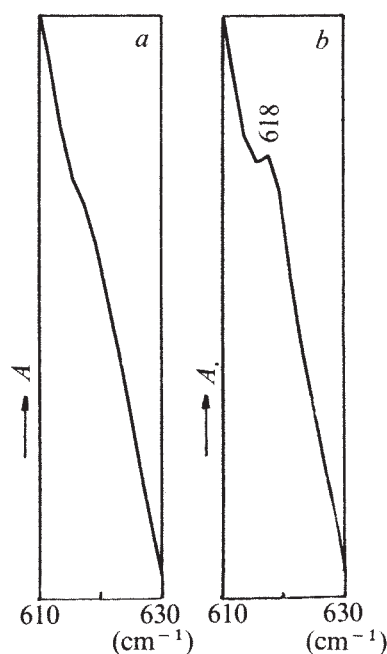
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Polymorphic transition in silica glass

THE short range order in glasses is now more or less generally accepted to have a dimension of 20 Å or slightly more^{1,2}. In the case of silica glass we have established³, on the strength of infrared absorption bands, that those regions (clusters, substructures) have the high-cristobalite structure, which is metastable at room temperature and normal pressure. It has been possible to relax a polymorphic transformation in pure silica glass into the stable low temperature form of cristobalite by heating a powdered sample at 483 K for 27 h (ref. 3). Heating relaxed the existing strains in the clusters by adjusting their structure to the low temperature conditions. A report by Wagstaff⁴ suggested another possible way of provoking the transition—by increasing the strains in the high-cristobalite clusters in silica glass by cooling it to liquid helium temperature.

Using the attenuated total reflectance (ATR) technique in infrared spectroscopy, we have proved that such a transformation takes place in experimental conditions, after a powdered sample of pure silica glass has been kept for several days at liquid helium temperature. No transition was observed in a piece of this glass kept in the same conditions, which agrees with the result of the heating experiments. The low-cristobalite form of silica shows a characteristic absorption band (618 cm⁻¹) which is absent in the regular high-cristobalite form³. This band provides an easy way of observing the transition process. Using powdered mixtures of pure silica glass and known quantities of crystalline low-cristobalite for an approximate calibration, we found about 1% conversion by weight of glass. This is a rather small amount of new phase compared with nearly 100% conversion reached in the heating experiments (but only in some of them). Nevertheless, we have obtained further evidence to confirm the possibility of provoking a

Fig. 1 ATR absorbance plots. *a*, Powdered silica glass before cooling at liquid helium temperature; *b*, the same sample after cooling.



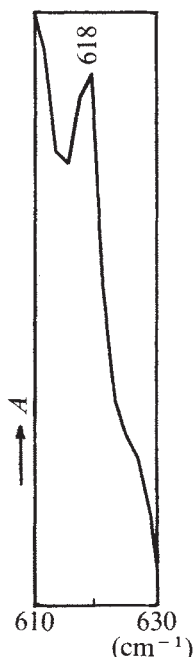


Fig. 2 ATR absorbance plot: computed ratio of Fig. 1a and b curves. All figures were drawn directly by the Digilab FTS-14 instrument.

polymorphic transition in X-ray amorphous substances, such as silica glass. Independent confirmation comes from the very sensitive thermal expansion observations on silica glass of C. F. Drake, referred to by C. H. Goodman⁵.

We are also studying the heat effects of the transition. Preliminary experiments with very sensitive differential scanning calorimetry equipment also unequivocally point to the presence of partial polymorphic transition in silica glass at 542K. This work is still in progress. Thus evidence is accumulating to suggest that polymorphic transitions occur in regions as small as about 20 Å. This, at least in the case of vitreous silica, has also helped to establish firmly the structure of clusters, as polymorphic transitions are strictly characteristic for each form of crystalline silica.

The Digilab FTS-14 Fourier transform spectroscopic instrument was used in this work and appropriate computer programs were used to compare spectra obtained before and after cooling the same sample of glass.

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Thermoluminescent dating of Lake Mungo geomagnetic polarity excursion

ARCHAEOMAGNETIC studies made by Barbetti and McElhinny^{1,2} on prehistoric aboriginal fireplaces at Lake Mungo, Australia, indicate the occurrence of a polarity excursion about 30,000 years ago during which the geomagnetic field intensity increased to three times its present-

day value; this main excursion was followed by a second one during which the intensity decreased to about one third of the present day value. This note reports thermoluminescent (TL) dating measurements that were carried out on fireplace material at the request of Dr M. F. Barbetti. Comparison of the TL dating with the radiocarbon ages^{3,4} is of particular interest because geomagnetic intensity variations may be the dominant cause of long term distortion of the radiocarbon time scale⁵⁻⁷. The geomagnetic field gives the Earth partial shielding from cosmic rays, particularly from the lower energy, component that reaches only the upper atmosphere and is responsible for most of the radiocarbon production. If the geomagnetic intensity was lower than usual, then the production rate would have been higher and this would cause relevant radiocarbon dates to be too recent. In this comparison of TL and radiocarbon ages we have not, however, found evidence for a disturbance of the radiocarbon time scale caused by geomagnetic polarity excursions.

The samples were baked clay-sand ovenstones from fireplaces F6, F7, F8, F9, F12 and F14 (see refs 1 and 2 for description and location of these hearths and ovens). Initially we attempted to use the fine-grain dating technique⁸. Apparent ages in the range 30,000-50,000 yr were obtained but these were considered to be a substantial overestimate due to the presence of ('spurious') TL (not induced by radiation) in the natural glow curve; this was presumably a result of the deposited carbonate that was evident in the samples. Attempts to eliminate the spurious TL by washing in acid were not successful.

The results reported here were obtained using quartz grains in the size range 90-125 µm, extracted from the baked clay-sand matrix by magnetic separation and sieving. The procedures followed were those developed by Fleming⁹ for dating quartz inclusions from pottery of the last two or three millennia, appropriately modified on account of the much greater age of the Lake Mungo fireplaces as outlined below and as will be discussed more fully elsewhere. After extraction the grains were etched in hydrofluoric acid in order to remove the outer layer of material that had been irradiated with alpha particles from uranium and thorium in the matrix. The natural TL, which was measured immediately after etching, is therefore that induced by the dose from beta, gamma and cosmic radiation received since the fireplaces were last used. The beta and gamma dose rates were evaluated from radioactive and chemical analyses of the samples and the surrounding material; a value of 15 mrad yr⁻¹ was used for the cosmic dose rate. The total dose received since last heating (the archaeological dose) was evaluated by comparison of the level of natural TL in the 375 °C region of the glow curve with the (natural+artificial) TL from a second portion, the artificial TL being that induced by irradiation with a ⁹⁰Sr-⁹⁰Y beta source. Studies of the TL growth characteristics of these samples indicated that it was not valid to make the assumption of linearity of TL against dose and an exponential fit to the data points was made instead of the usual linear fit; the exponential fit was of the form $(1 - \exp(-x/B))$, where x is the dose and B is a constant for which the experimentally determined average value for these samples was 40 krad.

Use of an exponential fit (rather than a linear one) gives archaeological doses, and hence ages, that are lower by about 18%. The primary reason for rejecting the use of a linear fit was the observation that $(G_2 - G_N)$ was on average 6% less than $2(G_1 - G_N)$, where G_N is the natural TL, and G_1 and G_2 are levels of TL from portions of sample to which beta doses had been administered before any heating of the sample, and the beta doses being 4.8 and 9.6 krad respectively. The inadequacy of the linear fit was further indicated by direct observation of TL growth with

Table 1 Thermoluminescence and radiocarbon dates of ovenstones from prehistoric fireplaces

Sample	Archaeological dose (krad)	Dose rate (rad yr ⁻¹)	Predicted errors (yr × 10 ⁻³)			TL date	¹⁴ C date
			Random	Systematic	Combined	(yr × 10 ⁻³)	(yr × 10 ⁻³ (5,568-yr half life))
First excursion							
F7B	7.05	0.20	3.6	4.2	5.6	35.3	
F7D	6.05	0.20	2.5	3.2	4.1	29.5	30.8 ± 0.5
F7P	6.50	0.21	4.4	3.7	5.6	31.3	—
F8B	7.95	0.21	2.7	5.7	6.4	37.9	28.3 ± 0.4
F9D	7.00	0.22	4.1	3.8	5.7	32.0	—
F9J	7.60	0.23	4.1	4.2	5.8	32.3	27.5 ± 0.3
F12E	6.25	0.16	6.2	4.4	7.7	38.6	28.0 ± 0.4
			Weighted mean (TL)		=	33.5 × 10 ³ yr	
			Standard error		=	1.3 × 10 ³ yr	
			Predicted error (random and systematic)		=	4.3 × 10 ³ yr	
			Average (¹⁴ C)		=	30.4 × 10 ³ yr (5,730-yr half life)	
Second excursion							
F6	5.50	0.25	2.8	2.4	3.7	21.8	26.3 ± 0.5
F14P	6.50	0.21	1.7	4.1	4.4	31.4	None

The predicted error is at the 68% level of confidence and calculated as explained in ref. 10. The TL ages were evaluated on the basis that the form of the TL growth characteristic was the same for all samples.

dose using portions from which the natural TL had been drained by heating. These 'second-glow' growth characteristics were exponential in form, having a value of B of about 10 krad. This is substantially less than the value of 40 krad that corresponds to an exponential interpretation of the 6% difference between $(G_2 - G_N)$ and $2(G_1 - G_N)$ mentioned above, but the higher value for B is preferred on the grounds that a change in growth characteristic often occurs when a sample is heated, particularly during the drainage of the natural TL.

The 6% difference between $(G_2 - G_N)$ and $2(G_1 - G_N)$ is interpreted in terms of an exponential rather than in terms of a binomial because the former corresponds theoretically to the trapping model in which electron concentration in the conduction band is proportional to the dose rate and the TL traps are limited in number; this is the next simplest model to the one that corresponds to linear growth. It is also to be noted that the binomial function predicts a maximum in the growth characteristic rather than saturation and, to the extent that the second-glow growth characteristic can be used as evidence, this is in strong contradiction with observation.

The data are given in Table 1. The measurement precision for these samples was rather poor and the scatter in the ages is not much greater than the scatter to be expected if the samples were all of the same age. Because of this an average TL age has been evaluated for the seven samples which are considered to be associated with the first polarity excursion on the basis of radiocarbon dating (that is, F7, F8, F9 and F12). Using our standard form of citation for TL dates the date obtained for the first excursion is 35,000 b.p. ($\pm 1,300$, $\pm 4,300$, OxTL 174a). The two error limits quoted, which are calculated on the usual basis^{10,11}, are respectively the standard error of the mean derived from the observed scatter of the individual ages, and the error predicted by taking into account all sources of uncertainty both random and systematic; the dominant contribution to the latter arises from uncertainty in the value of B .

This date is substantially more recent than the preliminary value quoted by Fleming¹² and it is not significantly different from the average of the radiocarbon dates that are available for these four fireplaces, having in mind that it is the second error limit that is relevant to this comparison, nor from the TL date of 30,400 b.p. quoted by Mortlock¹³ for fireplace F7.

As regards the second excursion it should be noted that the TL date obtained for fireplace F6 is substantially different from the average date obtained for the first excursion (having in mind that between TL dates for similar contexts it is the random error limit that is relevant) but that it differs by less than two standard deviations (of the overall TL error) from the two radiocarbon dates for that fireplace. Fireplace F14, undated by radiocarbon, was assumed to be associated with the second excursion on the basis of its low intensity value; however the TL result does not support this interpretation.

Although the present work gives no evidence that these geomagnetic polarity excursions gave rise to distortion of the radiocarbon time scale the data do not exclude a major perturbation; for example, the difference of 3,100 years between the average TL age and the average radiocarbon age for the first excursion, although within the TL error limit, could correspond to an atmospheric radiocarbon concentration 45% in excess of the level for AD 1850. However, the high geomagnetic field intensity associated with the first excursion, if global, would cause the atmospheric radiocarbon concentration to be in deficit, whereas it is only with the second excursion when the field was low that an excess would be expected.

We are grateful to Miss Sheridan Bowman for an important observation made in the course of subsidiary measurements on one of these samples and to the Nuffield Foundation for provision of equipment.

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Evolution of Lake Abhé (Ethiopia and TFAI), from 70,000 b.p.

WE summarise here the first continuous stratigraphic sequence for the late Quaternary of the African continent, derived from a detailed study of the evolution of an inter-tropical lake: Lake Abhé (11°N, 42°E) (Fig. 1). For the interval 20,000–0 b.p., this record complements and amplifies existing knowledge, and it also throws valuable new light on the preceding period. It provides evidence for important late Pleistocene climatic changes at the eastern end of the Sahel zone.

The results come from a multidisciplinary 5-yr project, and supporting detail is available elsewhere¹. Analyses of diatom associations, which are sensitive indicators of ecological conditions, were carried out on 492 samples, and supplemented by convergent evidence from sedimentary facies and mineralogy. Seventy-five ¹⁴C dates, derived from a 50-m core taken at the edge of the present lake (Fig. 1) and from stratigraphic sequences on the margin of the basin, provide a chronological framework with a high degree of reliability (Table 1).

Lake Abhé occupies a closed tectonic basin in the Afar (Fig. 1). The present lake (350 km²) has a surface elevation of 240 m, and is currently shrinking. Only a few metres deep, it is hyperalkaline and rich in sodium bicarbonate². Since it is situated at the downstream end of the perennial Awash River, which drops from a height of 2,500 m on the Ethiopian Plateau, the lake records climatic fluctuations in the Ethiopian highlands as well as in the Afar itself.

The main stages in the late Quaternary history of Lake Abhé are summarised in Fig. 2 and Table 1. Its Holocene record agrees in detail with findings from other parts of intertropical Africa^{3,4}. Three (possibly four) lacustrine phases are evident. The early Holocene phase, Abhé IV, is the most important. A comparable transgression has been

documented for several East African lakes^{1,5–9} and along the entire southern margin of the Sahara^{10–12} (Fig. 3). The Abhé IV sedimentary facies (Table 1) reflect very low turbidities, indicating that precipitation was abundant and evenly distributed through the year, as was the case throughout the Sahel zone^{11,13,14}. In spite of this parallelism in rainfall, early Holocene water temperatures deduced from the diatom floras seem to have been higher in Lake Abhé and in the rest of East Africa^{1,8,15} than in Chad and Mauritania (ref. 11 and B. Marciniak, personal communication). This may arise from the greater cooling of the Atlantic than the Indian Ocean during the late Wurm¹⁶. The brief but well defined regression ~8,000 b.p. in Lake Abhé can be connected with a fall in the level of Lakes Rudolf⁵, Nakuru⁵ and Chad¹¹ (Fig. 3) and with a change in the flow regime of the Blue Nile around 8,000–7,000 b.p. (ref. 10). It is followed by another transgression, Abhé V (Fig. 2) during which ecological conditions resembled Abhé IV although the deposits are more silty or clayey. The lake level began to fluctuate after 6,000 b.p. and fell rapidly from ~4,000 b.p. until the lake dried out. During late Holocene time, there were several minor fluctuations (Abhé VI, Abhé VII) but clastic deposits dominated. Productivity remained very low and has tended to zero during recent centuries. It is difficult to establish close correlations between Abhé VI or Abhé VII and the several minor humid phases apparent from other basins in intertropical Africa^{1,3,9,11,17}.

Data from Lake Abhé also confirm the existence of the hyperarid phase, synchronous with the European late Wurm, which affected intertropical Africa^{1,3,6,11,12,18–23} and probably the whole intertropical zone^{24–26} between 17,000 and 12,000 b.p. In the Abhé core, this arid episode is registered by a thick soil, while large debris fans formed on the basin flanks²⁷. Between 17,000 and 10,000 b.p. Lake Abhé was dry.

The most important results of the Lake Abhé study relate to the period before 17,000 b.p., about which little is known in intertropical Africa. Previously, only isolated dates

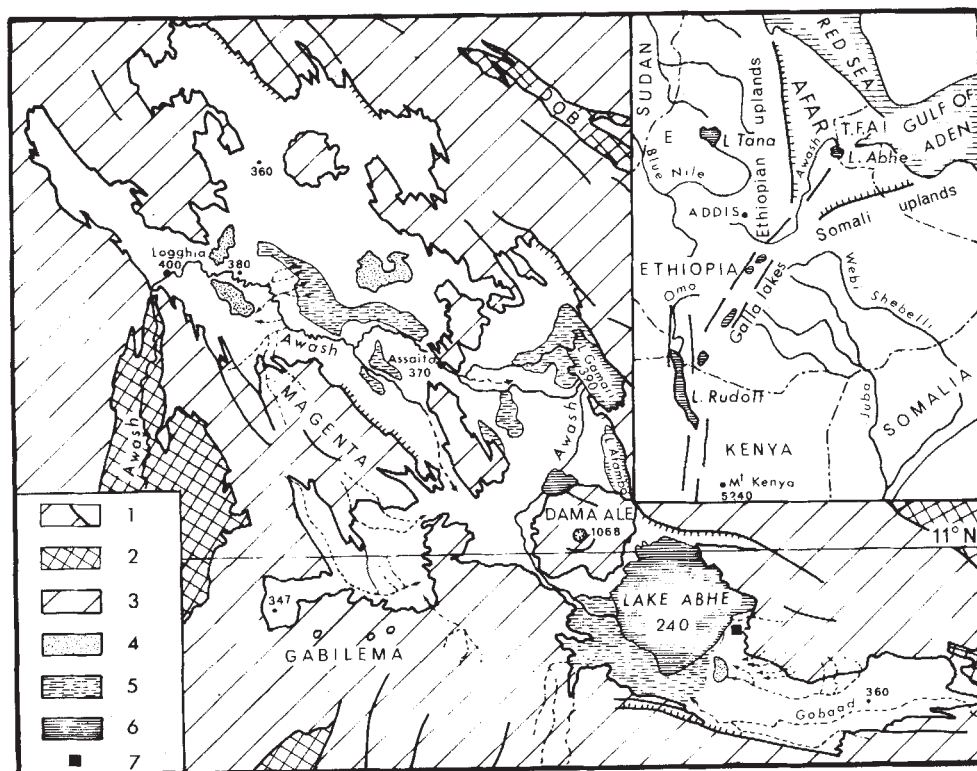


Fig. 1 The tectonic basin of Lake Abhé. 1, Edge of basin. Lake Abhé occupied almost all of this basin during the late Quaternary transgressive phases; 2, other tectonic basins; 3, basalt plateaux and volcanic massifs; 4, dunes; 5, swamps; 6, modern lake remnants; 7, borehole.

Fig. 1

Table 1 The main stages in the late Quaternary evolution of Lake Abhé

^{14}C Age (yr b.p.)	Climatic episode	Sedimentary facies	Characteristic diatoms	Salt content	pH	(SiO_2)	Temperature	Turbidity	Productivity
★ ■ 110 ± 50	Modern	Clay	None	P (160) (g/l)	S (10,3)	S (416) (mg/l)	T (28°C)	S	11
★ ● 1,030 ± 100	ABHE VII	Clay CaCO_3 (littoral)	None	M	S	S	T	S	1
★ ■ 1,570 ± 60	ABHE VI	Clay CaCO_3 (littoral)	<i>Stephanodiscus astraea</i> + var.	O	M	1	T	S	M
★ ■ 2,720 ± 120		Cobble-gravel							
● ● 4,120 ± 110	ABHE V	Clay, Silt	<i>Campylodiscus clypeus</i> , <i>Rhopalodia giberrula</i>	M, P			T	S	1
● ● 6,020 ± 160		Clayey CaCO_3	<i>Stephanodiscus astraea</i> var. <i>intermedia</i> + <i>minutula</i>	O	M	1	T	M	S
● ● 7,190 ± 220		Clay	<i>Cyclotella striata</i> , <i>Melosira agassizii</i>	M	M	S	T	S	1
● ● 7,610 ± 140		Cobble-gravel							
● ● 8,340 ± 180	ABHE IV	Clay	None						
● ● 8,450 ± 190		Pure CaCO_3	<i>Stephanodiscus astraea</i> var. <i>intermedia</i> + <i>minutula</i>	O	M	1	T	11	S
★ ● 9,380 ± 110		Clay	<i>Cyclotella striata</i> , <i>Melosira agassizii</i>	M	M	S	T	S	1
● ▲ 16,400 ± 800	LATE WURM	Paleosol (Abhe core)							
● ▲ 16,100 ± 700		Boulder-gravel							
● ■ 17,100 ± 400	ABHE III	CaCO_3 Calcareous silt	<i>Rhopalodia giberrula</i> + var., <i>Diploneis elliptica</i> , <i>Stephanodiscus astraea</i> var. <i>intermedia</i>	E	M	M		S	1
● ● 20,800 ± 400		Clayey calcareous diatomite	<i>Melosira granulata</i> var. <i>valida</i> , <i>M. nyassensis</i> + var. <i>victoriae</i> , <i>M. goetzeana</i>	O	M	S	T, t	S	M
● ● 23,190 ± 170		Silty diatomite	<i>Cyclotella ocellata</i> , <i>Melosira granulata</i>	O	M		t	S	1
● ● 23,560 ± 740		Gravelly diatomite	<i>Coscinodiscus faurii</i>	M				S	1
● ■ 23,900 ± 700		Gypsum (littoral)							
● ● 24,800 ± 650	ABHE II	Calcareous diatomite	<i>Cyclotella ocellata</i> , <i>Melosira granulata</i> , <i>Stephanodiscus astraea</i> var. <i>intermedia</i>	O	M	M	t	S	1
● ● 25,100 ± 700		Pure diatomite	<i>Nitzschia aequalis</i> , <i>N. stricta</i> , <i>N. nyassensis</i> , <i>N. subacicularis</i> , <i>Stephanodiscus hantzschii</i> var. <i>pusilla</i> , <i>Synedra rumpens</i> var. <i>neogena</i>	O	S	S	T >23°C	11	S
● ● 26,900 ± 700		Calcareous diatomite	<i>Nitzschia aequalis</i> , <i>N. stricta</i> , <i>Stephanodiscus astraea</i> , <i>S. hantzschii</i> var. <i>pusilla</i>	O	S	S		M	M
● ■ 30,600 ± 1700	EARLY WURM	Fluvial-sand (Abhe core)							
● ● 31,000 ± 1200		Pebble-gravel							
● ● 31,000 ± 1700	ABHE I	Sandy and calcareous clay	<i>Stephanodiscus astraea</i> + var.	O	M	M		S	M
● ● 34,400 ± 2100		Clay	<i>Nitzschia aequalis</i> , <i>N. stricta</i>	O	S	S	T	S	M
● ■ 35,000 ± 2900		Silt (littoral)	<i>Stephanodiscus astraea</i> var. <i>intermedia</i> + <i>minutula</i>	E	M	1		S	M
● ■ 35,000 ± 2900		Silty CaCO_3							
● ■ 40,000									
60,000?									
70,000?									

^{14}C -Age b.p.: only most important dates marking environmental changes are shown for the Holocene. All available dates are given for the late Pleistocene. Material dated: ●, shells; ■, well crystallised calcite or Mg-calcite; ▲, organic matter by; star in circle, G. Delibrias, Gif-sur-Yvette; ★, J. C. Fontes, Paris: open star, ref. 30; star in square, ref. 31.

Dissolved salt content: O, oligohalobe (< 2 g l⁻¹); M, mesohalobe (2 < 15 g l⁻¹); P, polyhalobe (> 15 g l⁻¹); E, fluctuating salinity.

pH: M, moderately alkaline; S, strongly alkaline.

(SiO_2), 1: < 10 mg l⁻¹; S: > 10 mg l⁻¹.

Temperature: T, tropical; t, temperate.

Turbidity and productivity: S, strong; M, moderate; 1, low; 11, very low.

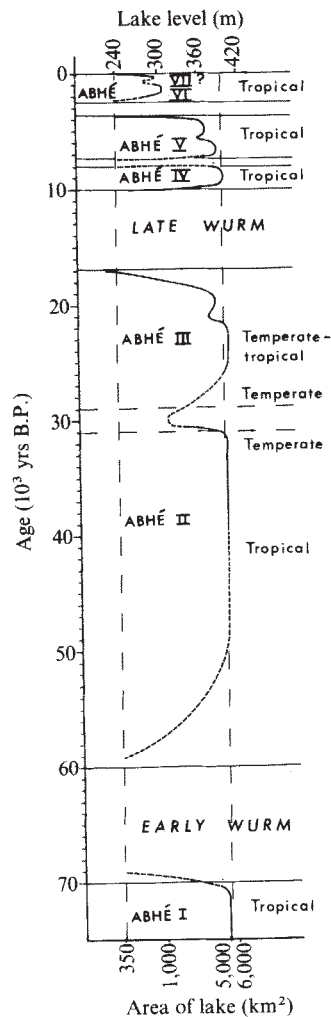


Fig. 2 Oscillations in the level of Lake Abhé from late Pleistocene to Holocene times. Based on 45 radiocarbon dates of littoral deposits for the period 0 to 35,000 b.p., and on extrapolation of mean sedimentation rates measured in the dated section of the core for the period before 35,000 b.p.

indicated high lake levels between 20,000 and 40,000 b.p. in Lake Nakuru⁵, Chad¹¹ and Mauritania¹². Three lacustrine phases (Abhé I, Abhé II, Abhé III), separated by drier episodes, occurred during the late Pleistocene. The earliest (Abhé I) lies beyond the range of ¹⁴C dating. By extrapolating from the upper part of the Abhé core, its base (50 m) is dated at 70,000–100,000 b.p. On the other hand, the dry phase between Abhé I and Abhé II strongly resembles the arid 17,000–10,000 b.p. interval, so that it may correspond to the early European Wurm, dated by extrapolation from deep-sea cores to 60,000–70,000 b.p. (ref. 28). Abhé II began before 40,000 b.p. and was terminated by a minor regression around 31,000–30,000 b.p. (Table 1). The third phase, Abhé III, is dated between 30,000–29,000 b.p. and 17,000 b.p.

These three lacustrine phases were of similar importance (Fig. 2) but they strongly differed in their physico-chemical attributes (Table 1). The Abhé I lake was warm, alkaline, and clastic sedimentation predominates. The succeeding arid phase is marked by fluvial sands and gravels in the Abhé core and by debris fans on its margins. Abhé II was an episode of biochemical sedimentation. The lake received only material in solution, which implies gentle, well distributed rain and/or a dense vegetation cover in the catchment area. The diatom flora indicates a very warm, transparent lake with high alkalinity and productivity. Synchronous with the minor regression towards 30,000 b.p., the change in sedimentary facies was accompanied by a clear decrease in water temperatures and productivity, registered by the spread of cosmopolitan and temperate species. During the phase Abhé III, clayey calcareous diatomite accumulated in the lake. Temperate conditions persisted until 25,000 b.p. But tropical diatoms, which could support cooler temperatures than the Abhé II flora, multiplied again between 25,000 b.p. and 20,000 b.p. The ecological conditions then began to fluctuate. Numerous carbonate horizons were precipitated and brackish flora developed. The lake dried up ~17,000 b.p.

The late Quaternary evolution of Lake Abhé reflects changing precipitation–evaporation patterns. It is not yet possible to disentangle the relative effects of temperature and precipitation changes during the lacustral phases. But the episodes of greatest cold were definitely characterised by

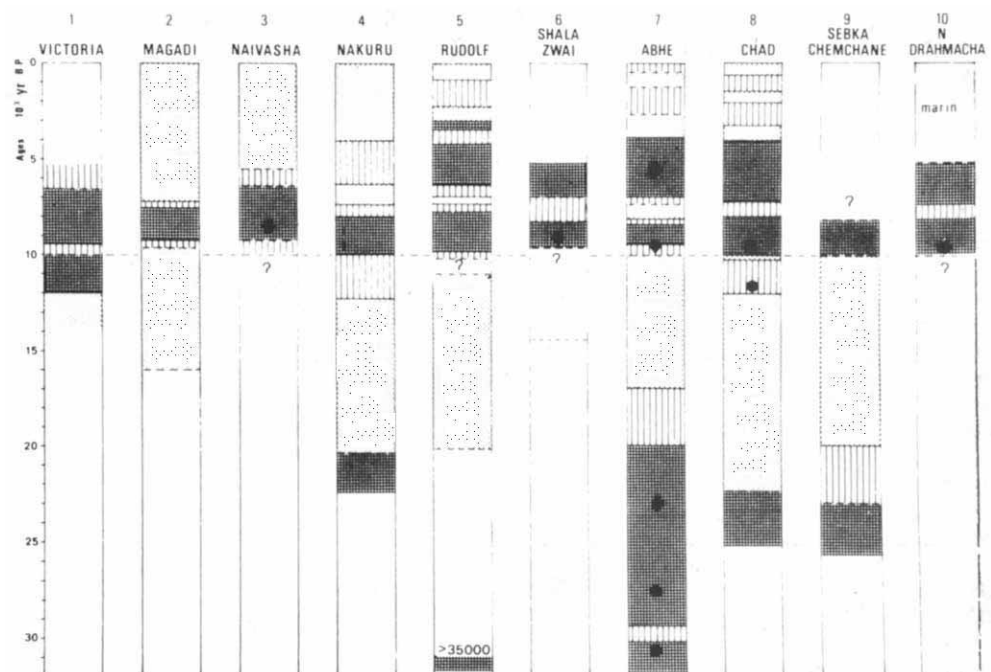


Fig. 3 Comparison between late Quaternary fluctuations of selected intertropical African lakes. From left to right the lakes grouped in order of increasing latitude (Lake Victoria 0°, Sebka Chemchane 21°N). (1) to (5) taken from ref. 5; (6) ref. 9; (7) this work; (8) ref. 11; (10) ref. 12 and B. Marciniak, personal communication. ♦, Diatom flora indicating a climate at least as warm as the present day; ●, diatom flora indicating a climate cooler than the present day.

aridity and lake-level minima. It is clear that fluctuations in the tropical atmosphere were systematically related to the high latitude glacial-deglacial sequence.

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Sulphur isotope abundance elucidate uptake of atmospheric sulphur emissions by vegetation

THE sour gas wells of Alberta form the basis of its sulphur industry. The well known Claus process is used, whereby part of the H_2S is oxidised to SO_2 which is in turn reacted with more H_2S to yield elemental sulphur. As a consequence, more than 1,200 t of sulphur per day are discharged into the atmosphere in Alberta, mostly as SO_2 . Using sulphur isotope abundances, I have assessed the uptake of these emissions by vegetation.

The H_2S processed in Alberta is typically enriched in the heavier ^{34}S with $\delta^{34}S$ values ranging from +5 to +30‰ (refs 1, 2) where $\delta^{34}S$ is defined in terms of the $^{34}S/^{32}S$ abundance ratio in meteoritic troilite as

$$\delta^{34}S(\text{‰}) = \left\{ \frac{[^{34}S/^{32}S]_{\text{sample}}}{[^{34}S/^{32}S]_{\text{meteorite}}} - 1 \right\} \times 1,000$$

In contrast, most Alberta soils have $\delta^{34}S$ values ranging from near 0 to -30‰ (ref. 3). Since these variations are many times the measurement precision ($\pm 0.2\text{‰}$ or better), sulphur isotope abundances can be used effectively to assess the impact of the sulphur emissions on the Alberta environment.

The success of this approach is illustrated by data from the Ram River Area in Alberta where two sour gas processing plants are operational (Fig. 1). H_2S is recovered from at least two geological reservoirs by the industrial operations and the stack SO_2 has $\delta^{34}S$ values that vary from +20 to +25‰. The major peak in the $\delta^{34}S$ distribution for air in Fig. 1 thus corresponds to the industrial emissions. At a given sampling site, the atmospheric isotopic composition depends on the ratio of industrial to background sulphur oxides. The latter vary considerably in concentration and isotopic composition depending on the season,

biological activity and contributions from other anthropogenic sources. The mixing ratios depend on site location and meteorological conditions. For these reasons, the histogram for air in Fig. 1 is not a sharp peak corresponding solely to the industrial emissions. The secondary peak in Fig. 1 near +11‰ will be discussed later.

Lichens (*Usnea* sp., Fig. 1) had an isotopic distribution which coincided closely with the major peak of the air. This shows that lichens take up sulphur from the air by direct pathways. One process is the interaction with the gaseous sulphur oxides. Another source is rain which has passed through sulphate formed by oxidation of the atmospheric SO_2 or dissolved particulate sulphate deposited on bark. Subsequent studies (unpublished) with *Usnea* and other genera have shown that interspecies isotopic variations are not greater than the range encountered within one species at a given site.

Upper mossy layers of the soil in this region have $\delta^{34}S$ values near +15‰, suggesting a considerable uptake of industrial emissions. Below this mossy layer (~1 cm), soil sulphur has $\delta^{34}S$ values near +2‰ indicating that moss effectively blocks the passage of sulphur compounds to the subsoil. Isotopic profiles from several soil types suggest that factors which promote penetration are lack of biological cover, net drainage and the length of time that the industrial source has been operational.

Although Fig. 1 shows that pine needles (*Pinus contorta*) had a sulphur isotope distribution of similar shape, this is shifted by

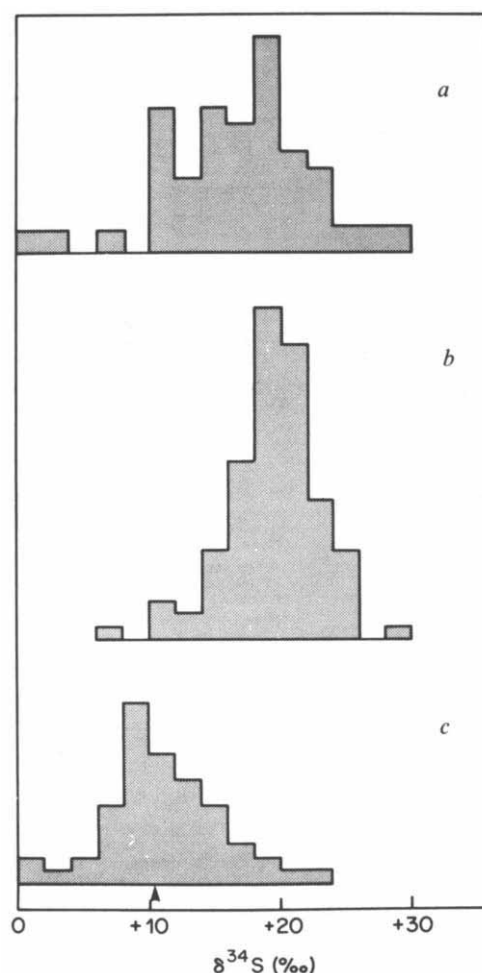


Fig. 1 Sulphur isotope variations in *a*, air; *b*, lichens and *c*, pine needles of the Ram River area for 1972-73. Atmospheric data were obtained from lead peroxide cylinders which had been set out for periods of 1 month to trap chemically the gaseous sulphur oxides. Lichens and live pine needles were gathered near the sites of the exposure cylinders. Their sulphur isotope data are based on all sulphur remaining after a brief ultrasonic washing with distilled water.

some 10‰ to lighter $\delta^{34}\text{S}$ values compared with those of air and lichens. In contrast to lichens and mosses, whose rhizines and rhizoids serve mainly for attachment, trees have root systems which transport sulphate upwards. Pine needles derive sulphur from both soil ($\delta^{34}\text{S} \sim +2\text{‰}$) and air (heavier $\delta^{34}\text{S}$ values) and therefore intermediate $\delta^{34}\text{S}$ values arise. Thus it might be argued that the data are not unexpected. But the isotopic technique facilitates assessment of the proportions of sulphur derived from the two sources. More recent data from a control site in the Ram River area, where the effects of the emissions were minimal, gave $\delta^{34}\text{S}$ values for needles and leaves similar to those of the soil ($\sim +2\text{‰}$). In another situation, sulphur compounds which originated with the industry had penetrated the soil to at least 40 cm and the conifer needles had $\delta^{34}\text{S}$ values near those of the ambient SO_2 . At a given location under the influence of sulphur emissions, pine needles tend to have higher $\delta^{34}\text{S}$ values than spruce, indicating that the former derive proportionately more sulphur from the atmosphere. In the case of a very high *Pinus contorta* (~ 24 m) close to a sour gas processing plant (near Whitecourt, Alberta) an increase in $\delta^{34}\text{S}$ with height has been found, indicating that the canopy shields the undergrowth from the emissions.

Plots of $\delta^{34}\text{S}$ for lichens and needles compared with $\delta^{34}\text{S}$ values for atmospheric SO_2 are not as valuable for drawing conclusions as the histograms of Fig. 1. The chief reason is that the atmospheric data represent monthly averages whereas the times associated with the uptake of sulphur by lichens and needles are unknown. When certain growths can be correlated to a given period, there is no assurance that the rate of sulphur intake was uniform throughout that time. For these reasons my study lasted 2 yr in the Ram River area and needles and lichens were picked randomly near the air-monitoring stations. One concern is the extent to which the exposure cylinders are selective in the isotopes they take up especially because all field conditions are not readily simulated in the laboratory. Data obtained with high volume atmosphere sampling techniques under the auspices of the University of Calgary Interdisciplinary Sulphur Research Group (UNISUL) have been generally consistent with those obtained with lead peroxide cylinders (unpublished results). Although some of the data in Fig. 1 might have been shifted in extreme field conditions, lack of confidence in the more frequent values seems unjustified. In that the most frequent $\delta^{34}\text{S}$ values for the exposure cylinders and the lichens coincide, it would be necessary to postulate similar isotopic selectivities for both uptake mechanisms. This seems unlikely.

Several questions remain concerning the general mobility of sulphur compounds in vegetation. Pine needles can release sulphur compounds to the air and this may account for the secondary peak at $+11\text{‰}$ for the atmospheric data of Fig. 1, which almost coincide with the peak in the needle data. Another problem is the extent to which isotopic selectivity is involved in the uptake and release of sulphur compounds by trees. These problems are being investigated. This study was supported by the Aquitaine Company, the Atmospheric Environment Service of Environment Canada and the Canadian Forestry Service.

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haemoglobins in these populations. The generally accepted view is that the maintenance of balanced polymorphism in these populations at the loci of the commonest hereditary erythrocyte defects is due to a lesser susceptibility of the heterozygote²⁻⁷. An examination of that claim using a mathematical model shows that polygamy can lead to an equilibrium distribution in gene frequencies only if the male heterozygote tends to acquire more wives than either homozygote—a condition that is shown not to be unlikely in African and Islamic populations.

We consider a pair of alleles A_1 and A_2 and suppose that the associated genotypes A_1A_1 , A_1A_2 and A_2A_2 have frequencies Q_{11} , Q_{12} and Q_{22} , respectively, in the present population before selection ($Q_{11} + Q_{12} + Q_{22} = 1$). We assume the population is so large that the effect of random fluctuation of gene frequencies may be neglected. The generations are non-overlapping. Suppose s_1 and s_2 are the premating selection coefficients against the homozygotes A_1A_1 and A_2A_2 , respectively ($0 \leq s_1, s_2 \leq 1$), then the premating genotype frequencies (after selection) are

$$q_{11} = \frac{(1-s_1)Q_{11}}{W}, \quad q_{12} = \frac{Q_{12}}{W}, \quad q_{22} = \frac{(1-s_2)Q_{22}}{W}$$

for A_1A_1 , A_1A_2 and A_2A_2 , respectively, where W is a normalising constant chosen to make $q_{11} + q_{12} + q_{22} = 1$.

To incorporate polygamy we assume further that a man with genotype A_iA_j may marry n wives simultaneously or one after the other at random with probability

$$R_{ij}^{(n)} \left(\sum_n R_{ij}^{(n)} = 1 \right).$$

Let ${}_{ij}P_{rs}^{(n)}$ denote the conditional probability that an A_iA_j man, who marries n wives, will have an A_rA_s child. ${}_{ij}P_{rs}^{(1)}$ shall be shortened to ${}_{ij}P_{rs}$ for convenience. Then it follows from the condition that wives are chosen at random that

$${}_{ij}P_{rs}^{(n)} = 1 - (1 - {}_{ij}P_{rs})^n \quad (1)$$

$$\text{Pr}(A_iA_j \text{ man marries } n \text{ wives}) = q_{ij}R_{ij}^{(n)} \quad (2)$$

The zygotic frequencies (before selection) in the next generation are then found to be

$$\begin{aligned} Q'_{11} &= \frac{1}{T} \sum_{n=1}^{\infty} \left[q_{11}R_{11}^{(n)} {}_{11}P_{11}^{(n)} + q_{12}R_{12}^{(n)} {}_{12}P_{11}^{(n)} + q_{22}R_{22}^{(n)} {}_{22}P_{11}^{(n)} \right] \\ Q'_{12} &= \frac{1}{T} \sum_{n=1}^{\infty} \left[q_{11}R_{11}^{(n)} {}_{11}P_{12}^{(n)} + q_{12}R_{12}^{(n)} {}_{12}P_{12}^{(n)} + q_{22}R_{22}^{(n)} {}_{22}P_{12}^{(n)} \right] \\ Q'_{22} &= \frac{1}{T} \sum_{n=1}^{\infty} \left[q_{11}R_{11}^{(n)} {}_{11}P_{22}^{(n)} + q_{12}R_{12}^{(n)} {}_{12}P_{22}^{(n)} + q_{22}R_{22}^{(n)} {}_{22}P_{22}^{(n)} \right] \end{aligned} \quad (3)$$

where T is a normalising constant chosen to make $Q'_{11} + Q'_{12} + Q'_{22} = 1$. ${}_{ij}P_{rs}$ can be expressed in terms of these mating

Polygamy and genetic equilibrium

POLYGAMY is the normal practice in indigenous African and Islamic populations. Recently Konotey-Ahulu¹ has claimed that polygamy may explain the persistence of some abnormal

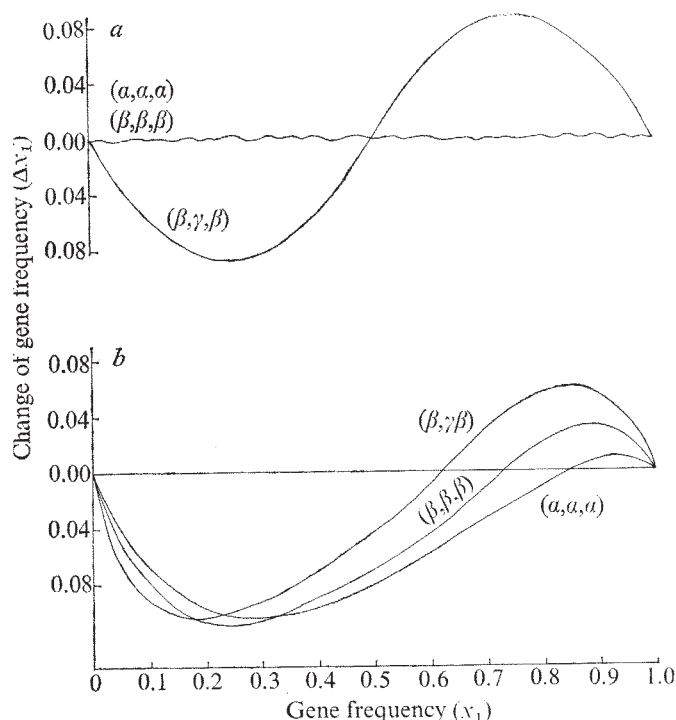


Fig. 1 Change in frequency of A_1 , ΔX_1 , at different values of the initial gene frequency X_1 . *a*, No premating selection and no inbreeding, that is $s_1 = s_2 = F = 0$. *b*, $s_1 = 0.2$, $s_2 = 0.6$, $F = 0.1$. A graph marked (β, γ, β) refers to the situation where the conditional probability distribution of the number of wives are β , γ and β for A_1A_1 , A_1A_2 and A_2A_2 men, respectively. α , β and γ are the hypothetical probability distributions below.

	Number of wives (<i>n</i>)								Total
		1	2	3	4	5	6	>6	
Probability distributions	α	1	0	0	0	0	0	0	1
	β	0.5	0.3	0.1	0.06	0.02	0.01	0.01	1
	γ	0.1	0.13	0.17	0.40	0.18	0.01	0.01	1

α : A_1A_1 is monogamous; β : Half A_1A_1 men marry one wife, the others marry more than one wife with decreasing probability; γ : Most A_1A_1 men marry more than one wife, four being the commonest number of wives.

Note: (β, γ, β) describes the gene frequency changes for the situation where A_1A_2 has a greater probability than A_1A_1 and A_2A_2 to acquire many wives.

frequencies as shown in Table 1. So we can write Q'_{ij} explicitly in terms of the Q_{ij} values. The set of equations (3) then specifies the relationship between the genotype frequencies of any generation and that of the preceding generation. The form of equation (3) indicates that polygamy will not affect the behaviour of the system unless $R_{11}^{(n)}$, $R_{12}^{(n)}$ and $R_{22}^{(n)}$ differ. General solutions to the system could not be found. Some of the results of numerical calculations are illustrated in Fig. 1.

We note (Fig. 1*a*) that when there is no premating selection and no inbreeding, and every man marries one wife, every point is at equilibrium. This is consistent with the Hardy-Weinberg equilibrium law that a large population under random mating reaches an equilibrium no matter what the initial allelic frequencies may be. Other cases showed that polygamy will not produce a stable equilibrium in the absence of premating selective forces, except for the cases where the heterozygotes tend to acquire more wives than the homozygotes.

Our model is obviously oversimplified, but it does show that where the male heterozygote has a greater tendency to acquire

more wives than either homozygote we can have non-trivial equilibrium at intermediate gene frequencies. This is in fact another form of differential selection in which heterozygotes are favoured over the homozygotes, and supports the hypothesis put forward by Fisher⁸ to explain the phenomenon of balanced polymorphism. In effect, then, our model is equivalent to one in which selection operates differently in males and females as is the case with Owen's and Mandel's generalisation of it⁹⁻¹¹. However, ours has the advantage of incorporating polygamy directly, and considers selection really in two stages: premating selection, and selection in the number of mates. This is important in the discussion on the maintenance of genes for abnormal haemoglobins in populations where malaria is endemic and polygamy is practised. Differential polygamy in which male heterozygotes show a greater fitness by virtue of their potentiality to acquire more wives, will enhance the effect of premating selective forces which favour the heterozygotes, in the maintenance of genetic variability in the population.

Table 1 Calculation of ijP_{rs}

Man	Offspring	Wife			ijP_{rs}
		A_1A_1 (q_{11})	A_1A_2 (q_{12})	A_2A_2 (q_{22})	
A_1A_1	A_1A_1	1	$\frac{1}{2}$	0	$q_{11} + \frac{1}{2}q_{12}$
	A_1A_2	0	$\frac{1}{2}$	1	$\frac{1}{2}q_{12} + q_{22}$
	A_2A_2	0	0	0	0
A_1A_2	A_1A_1	$\frac{1}{2}$	$\frac{1}{4}$	0	$\frac{1}{2}q_{11} + \frac{1}{4}q_{12}$
	A_1A_2	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}q_{11} + \frac{1}{2}q_{12} + \frac{1}{2}q_{22}$
	A_2A_2	0	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}q_{12} + \frac{1}{2}q_{22}$
A_2A_2	A_1A_1	0	0	0	0
	A_1A_2	1	$\frac{1}{2}$	0	$q_{11} + \frac{1}{2}q_{12}$
	A_2A_2	0	$\frac{1}{2}$	1	$\frac{1}{2}q_{12} + q_{22}$

In African and Islamic populations malaria is prevalent. The heterozygotes are therefore more likely to survive childhood than the normal homozygotes. These heterozygotes will tend to acquire more wives than the normal homozygotes because about half the children a heterozygous man has with a homozygous recessive woman will be homozygous for the unfavourable allele, and about one-quarter of the children he has with a woman who is heterozygous will be homozygous for the unfavourable allele. Most of the children homozygous for the unfavourable allele used to die very early and, since in African populations procreation is the *raison d'être* of marriage, the heterozygote would tend to marry more wives, in the hope that the children he gets with his new wives will 'stay'. Our analysis supports the view of Konotey-Ahulu¹ that polygamy could be one of the factors in the maintenance of the sickle gene in African and Islamic populations. It will enhance the effect of premating selective forces.

In our analysis we assumed hypothetical values for the probability that an A_1A_1 man would marry n wives. The Ghana Institute of Clinical Genetics, Accra, is collecting data that would enable us to estimate these probabilities in African populations.

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Lek behaviour in three species of the subgenus *Hirtodrosophila* of Australian *Drosophila*

LEK behaviour, or the use of courting territories, is well known in the patterned wing, Hawaiian species of the subgenus *Drosophila*, but not elsewhere in the genus¹. Australian *Drosophila* comprise a major adaptive radiation in the subgenus *Scaptodrosophila*, and minor radiations in *Hirtodrosophila* and *Sophophora*^{2,3}. We report here on three species of the subgenus *Hirtodrosophila*, two with patterned wings and one without, that show lek behaviour.

The species are found in rain forest habitats from eastern Victoria to northern Queensland, a latitude range of 37°S–16°S; floristic composition of the rain forests varies widely over this range⁴. In especially damp and shaded regions, however, bracket fungi (Polyporaceae) of various species occur, growing laterally from the sides of fallen logs or tree trunks close to the ground⁵. The three *Hirtodrosophila* species use the horizontal undersides of the fungi as leks. Males position themselves on the undersides of fungi at regular intervals; females from the surrounding vegetation occasionally land there, and courtship activities follow. The undersides of the fungi are white or light grey, strongly enhancing displays; flies have not been found on old fungi with dark undersides.

During courtship, wing display is prominent. Two species, *D. mycetophaga* and *D. polypori*, have patterned wings, and both are commonly found from eastern Victoria to southern Queensland (latitude 2°S)³. The third species, *D. mixtura*, however, occurs only in northern Queensland. Its wings are tinged brownish especially towards the costal margin, but in contrast to other Australian *Hirtodrosophila* species and the Hawaiian species, it is striking in appearance, being very dark above, and changing abruptly to unicolorous pale cream below. Like the patterned-wing species, therefore, flies stand out strikingly when displaying on the undersides of bracket fungi.

In addition, two other as yet undescribed and rarer species have been collected from under bracket fungi, one with patterned wings, and one very closely related to *D. mixtura*. Presumably, they too use the fungi as leks. It is remarkable that only three patterned-wing Australian *Hirtodrosophila* species are known, all apparently using the same defined courting territories.

The five species are somewhat larger than many other Australian endemic species³. In contrast to the Hawaiian patterned-wing species¹, sexual dimorphism has not evolved, except that males are somewhat smaller than females. A striking level of sexual dimorphism has presumably evolved in the Hawaiian species, because the advertising males occupy sites in foliage to attract females to these seemingly less well defined courting territories.

We conclude that two patterned-wing species in the southern part of Australia, and a non patterned-wing species in the north show lek behaviour, the leks being identical in type. Moreover Hawaiian lek species belong to the subgenus *Drosophila* suggesting that convergent evolution has occurred in two subgenera of the genus for a behavioural pattern of considerable evolutionary significance which is unreported elsewhere in the genus.

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Longevity and lifetime body weight in mice selected for rapid growth

AN association between lifespan and early growth rate as affected by nutritional manipulation has been reported; high intake–rapid growth apparently contribute to shorter lifespan, and low intake–slow growth to longer lifespan^{1,2}. Genetically determined rapid growth has also been reported to be associated with shortened lifespan³.

A strain of mice with approximately double normal growth rate, and with modified food intake, body composition and reproduction patterns has been developed by selection for body weight gain from 21–42 d of age⁴. We report here data on the lifespan and lifetime body weight of this strain (G) developed by us, and of contemporary animals from an unselected random bred strain (C) from the same base population.

The study involved 30 litters of line C and 38 litters of line G, from generation 33 of both lines. Two mice of each sex were sampled from each litter, and weighed weekly beginning the day after birth, to 16 weeks. From weaning, at three weeks, they were housed four to a cage, by sex and strain. At 16 weeks, numbers were reduced by random sampling within litters to a total of 16 per sex per strain, representing 30 and 32 litters for C and G respectively, and the number per cage was reduced to two. 'White diet', with a guaranteed analysis of not less than 24% crude protein and 6% fat, and not more than 3.5% crude fibre, was fed *ad libitum* throughout the experiment.

The 64 animals selected at 16 weeks were weighed at 4-week intervals until they died from 'natural' causes. An autopsy was carried out on the majority of the animals, and the incidence and location of tumours and other abnormalities noted.

Mean life span for both lines, by sex, is given in Table 1. On average, animals from line G had a life span only 57% that of those from line C.

Table 1 Mean lifespan (weeks) for two strains of mice: control (C) and rapid 3–6-week body weight gain (G)

Line	Males		Females	
	<i>n</i>	$\bar{X} \pm \text{s.e.}$	<i>n</i>	$\bar{X} \pm \text{s.e.}$
C	16	112.1 $\pm$ 5.4	16	119.5 $\pm$ 3.8
G	16	51.1 $\pm$ 5.7	16	82.3 $\pm$ 3.2

Since all animals of both strains in this study had the same diet, the results are in agreement with the finding¹ that growth rate is more important than dietary composition in influence on lifespan.

There was a significant interaction between line and sex in that females in line G lived about 60% longer than males, while the two sexes in line C did not differ significantly in longevity.

Previous work with these strains had shown that line G mice have significantly higher food intake and body fat percentage than line C^{5–8}. Based on data from nutritional

manipulation experiments with rats^{1,2}, it seems probable that the premature ageing in line G is associated with obesity.

A variety of tumours, in terms of tissues and sites, was observed at autopsy; a pattern already noted in rats². The numbers of animals found to have tumours when autopsied was similar in the two sexes, and non-significantly higher in line G (48%) than in line C (39%). Since the life span in line G was considerably shorter than that in line C, it seems that tumours develop earlier in line G, and probably more frequently. A relation between tumour risk and calorie intake in rats is known to exist⁹.

Mean lifetime body weights for both lines and both sexes are shown in Fig. 1. The number of animals for determination of the means decreased with time, and thus the reliability of the estimates of line mean weights

suggests that it may be a particularly useful model for studying causal relationships between food intake, growth, obesity, and ageing.

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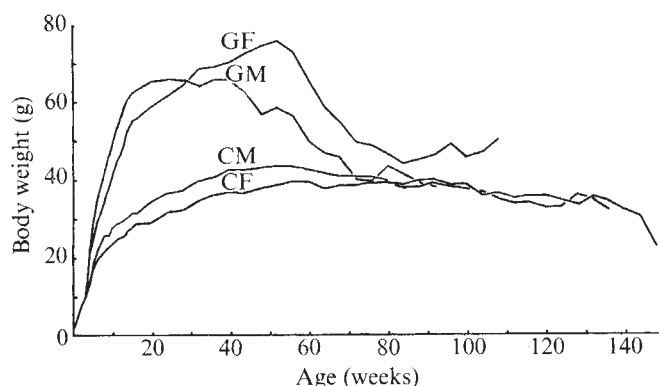


Fig. 1 Mean body weight (g) plotted against age (weeks) for males and females of two lines of mice: control (CM and CF) and rapid growth (GM and GF).

becomes progressively less. However, lifetime growth curves for the later surviving mice were very similar to those shown for all mice, and the difference between the two strains in growth pattern is clear. Selection for rapid 3-6-week gain resulted in a considerably higher mature weight in line G than in line C. Line G mice attained maximum weight at an earlier age than those in line C, and then promptly declined, to a value about 40% below the maximum and near that of the control. In both lines, males attained maximum weights at earlier ages than did females.

Roberts³ studied longevity in mice selected for increased body weight. All animals were mated in that experiment, so the results on longevity cannot be compared with those in this study, at least with regard to females, and possibly also not for males¹⁰. Roberts' results are, however, very similar to ours with regard to the earlier attainment of maximum body weight in animals selected for rapid growth, followed by a marked decline in weight beginning shortly after attainment of maximum weight and continuing until death. This decline he attributed to a depletion of fat reserves, also indicated by the appearance of our mice.

Experimental studies of longevity are of necessity costly, and time consuming. Male mice of strain G described here had a mean lifespan, under good husbandry conditions, of just under one year, which may represent the shortest lifespan available in a mammalian species readily available for experimentation. This, coupled with the fact that the strain is known to differ from the the unselected control in food intake, body composition, food conversion and certain other physiological parameters, and that the pattern of tumour incidence at death is apparently quite similar to that in rats with nutritionally induced variation in lifespan,

Naloxone-induced inhibition of ethanol dependence in mice

It has been suggested that tetrahydroisoquinoline (TIQ) alkaloids formed *in vivo* after ethanol ingestion represent metabolic sequelae linking the types of addiction caused by ethanol and the opiates^{1,2}. The theory derives from the fact that biosynthesis of opiate alkaloids involves TIQ intermediates³, and that TIQ formation from β -arylalkylamines and aldehydes proceeds rapidly at physiological pH (ref. 4). There have been strong criticisms of this hypothesis⁵⁻⁷, but the demonstration of an effect on opiate activity by certain neuroamine-derived TIQs⁸⁻¹⁰ prompted us to investigate the question further. Specifically, it was interesting to know the effect of naloxone, an agent known to block the development of dependence to opiates¹¹, on the development of dependence on ethanol.

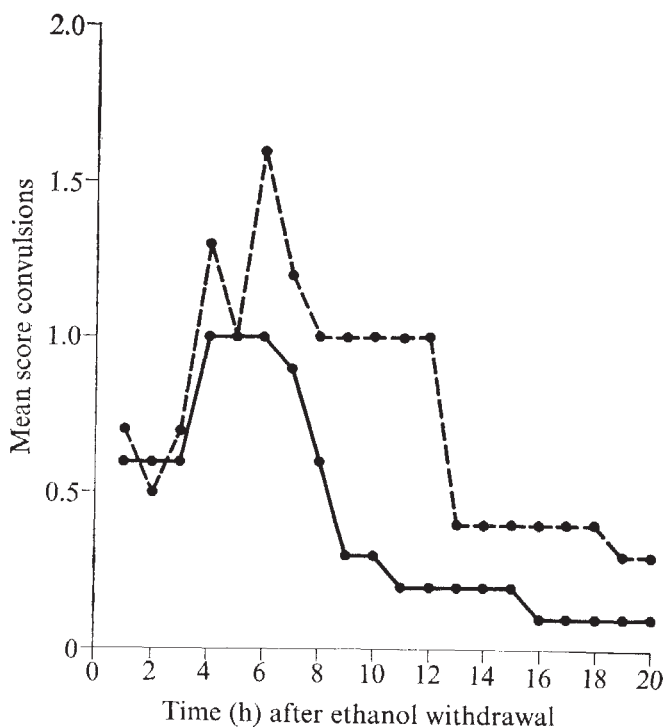


Fig. 1 Effects of chronic naloxone injection of the ethanol-induced withdrawal response in mice (—). Naloxone (2 mg kg⁻¹) was injected intraperitoneally every 6 h for 1 d before exposure to ethanol and every 6 h for 3 d during exposure for a total of 16 injections. Control mice (---) were injected with saline at the same time and with the same volume as the naloxone-treated mice. At least ten mice were used in each group.

Physical dependence on ethanol was induced in male Swiss-Webster mice (18–25g) by the technique of Goldstein and Pal¹². Groups of 24 mice were housed in airtight chambers and exposed to ethanol vapour carried by a slow stream of air for 3 d. Daily throughout the experimental period, concentrations of ethanol in blood and air were determined¹⁴. The animals received either naloxone (2 mg kg⁻¹ in saline) or equivalent volumes of saline every 6 h for 1 d before exposure to ethanol vapour, and every 6 h for 3 d during exposure. A priming dose of ethanol (1.67 g kg⁻¹, 25% v/v) and pyrazole (68 mg kg⁻¹), a compound known to inhibit ethanol metabolism and thus ensure stable blood ethanol concentrations¹³, was injected intraperitoneally on the first day of exposure to ethanol. Drug doses were calculated as the base form rather than the salt. On the second and third days, the mice were removed from the chamber once a day for 45 min for collection of tail blood samples and the single daily injection of pyrazole, and every 6 h for injections of naloxone or saline. Withdrawal was induced on the fourth day by removal of the mice from the ethanol chambers. Withdrawal severity, as measured by convulsions on handling has been described. Pyrazole control experiments, in which pyrazole was injected daily (68 mg kg⁻¹) without ethanol vapour, produced no significant convulsions when the drug was withdrawn on the fourth day.

The 6-h injections of naloxone significantly inhibited ($P < 0.01$) convulsions in response to withdrawal of ethanol. Scores were lower (14 times) at peak differences than those of the paired saline control. The mean convulsion score for saline

was 0.76 ± 0.05 , whereas that for naloxone was 0.46 ± 0.04 ($P < 0.01$), showing marked inhibition of ethanol dependence in mice (Fig. 1).

To illustrate this further, a composite of three experiments with a total of 72 mice is represented as a histogram in Fig. 2. The experimental protocol was the same as that described above. Naloxone (2 mg kg⁻¹), administered in four divided doses, reduced the withdrawal convulsions consistently and reproducibly in triplicate experiments. We calculated a ratio representing withdrawal severity (WS) according to the following formula

$$WS = \frac{\text{saline withdrawal score} - \text{naloxone withdrawal score}}{\text{naloxone withdrawal score}}$$

$$\left[\frac{(S-N)}{N} \right]$$

When $WS > 0$, the withdrawal convulsions have been inhibited; when $WS < 0$, the convulsions have been exacerbated. Fig. 2 shows the percentage inhibition (or excitation) of WS by naloxone during withdrawal from ethanol.

To analyse ethanol in the blood and brain of mice undergoing withdrawal from ethanol vapour, two groups were injected with either saline (equivalent volume as naloxone) or naloxone (2 mg kg⁻¹) every 6 h for 4 d, three of which were during exposure to ethanol. These mice also received daily injections (68 mg kg⁻¹) of pyrazole during exposure, as before. Blood and brain samples were taken from these groups 5, 10, and 15 h after withdrawal. Mice were killed and blood and brain samples from each were analysed for ethanol by gas chromatography¹⁴. At least five mice were used for each determination.

Ten hours after withdrawal (the time of peak seizure scores) the concentrations of ethanol in the blood or brain after administration of saline were 41.5 ± 3 mg dl⁻¹ and 35.7 ± 4 mg dl⁻¹, respectively; these values do not differ significantly from those obtained after administration of naloxone, which were 47.5 ± 2 mg dl⁻¹ and 43.7 ± 2 mg dl⁻¹, respectively. Similarly, naloxone did not affect the concentrations of ethanol at 5 h after withdrawal. Thus, the effect of naloxone on convulsions induced by withdrawal of ethanol do not seem to be related to an alteration of ethanol metabolism.

We next analysed brain calcium in mice given naloxone or saline and then exposed to ethanol vapour using the modified wet ash procedure of Cardenas and Ross²¹.

Mice were divided into two groups of twelve. Group A received an injection of naloxone (2 mg kg⁻¹) every 6 h, and group B received an equivalent volume of saline. They were treated 24 h before exposure to ethanol. The injection schedule continued daily for 3 d before withdrawal convulsions were recorded. Pyrazole (68 mg kg⁻¹) was injected as before.

Table 1 shows that chronic exposure to ethanol produces a significant decrease ($P < 0.01$) in brain calcium, to 70% of control in the first 24 h. On the second and third day of ethanol exposure, the effect was similarly significant ($P < 0.05$), though less effective, depleting calcium to 78 and 84% of control values, respectively. Naloxone, which by itself has no significant effect on brain calcium levels, prevented the ethanol-induced depletion of brain calcium ($P < 0.05$). These results suggest that the effect of naloxone on ethanol-induced withdrawal convulsions is mediated, at least in part, through an action on calcium metabolism in the central nervous system.

The finding⁷ that naloxone, an agent known to precipitate jumping in morphine-tolerant mice¹⁵, does not have this effect when administered acutely to ethanol-tolerant mice, cast doubt on the alkaloid biogenesis theory linking opiate and ethanol actions. Nonetheless, several lines of research have produced evidence that argues for such a relationship. Thus, cross-tolerance between morphine and ethanol¹⁶, increased tolerance

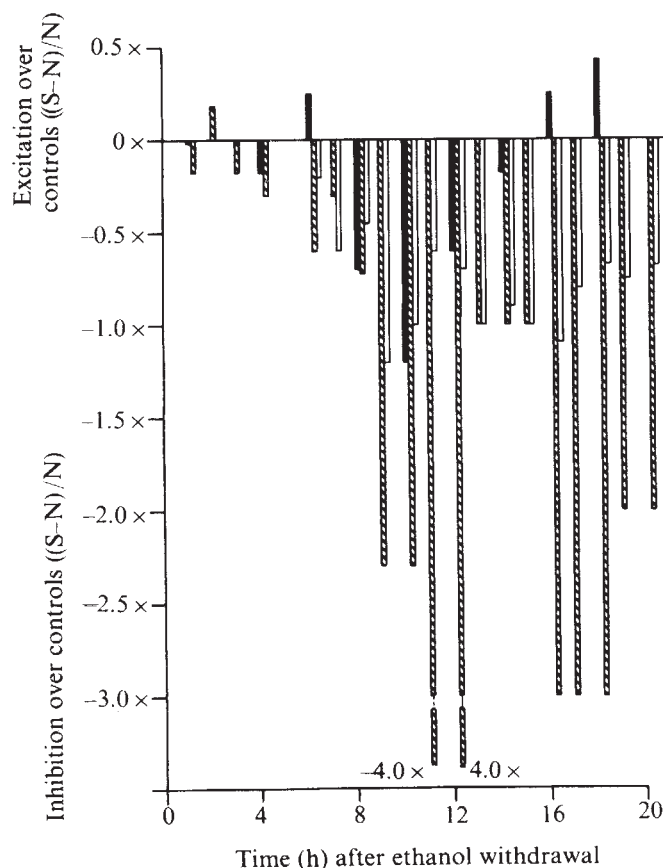


Fig. 2 Effects of naloxone on ethanol withdrawal convulsions from each of three experiments. The fraction $((S-N)/N)$ represents withdrawal severity and is calculated from

$$\frac{\text{mean saline convulsions} - \text{mean naloxone convulsions}}{\text{mean naloxone convulsions}}$$

for each of the 20 h listed (see text). Twenty-four mice were used for each experiment. Open columns, experiment 1; hatched columns, experiment 2; filled columns, experiment 3.

Table 1 Naloxone blockade of chronic ethanol-induced depletion of brain calcium in mice

Treatment	Brain calcium during chronic ethanol exposure* (mg g ⁻¹ (mean $\pm$ s.e.m.) wet weight)		
	Day 1	Day 2	Day 3
Saline (equiv. volume to naloxone + ethanol vapour)	36.0 $\pm$ 4.8†	39.9 $\pm$ 0.3†	43.2 $\pm$ 1.6†
Naloxone + ethanol vapour naloxone was administered at 2 mg kg ⁻¹ every 6 h (24 h before and every day during ethanol vapour exposure)	51.0 $\pm$ 2.6‡	45.8 $\pm$ 3.8‡	45.1 $\pm$ 4.8‡

Ten mice were used for each determination.

Calcium value for control brain = 51.0 $\pm$ 6.3 and for naloxone (2 mg kg⁻¹) = 52.2 $\pm$ 2.0.

* Mice were killed 24 h apart (day 1 = 24 h exposure, day 2 = 48 h and day 3 = 72 h).

† Values significantly different from controls ($P < 0.05$).

‡ Values not significantly different from controls.

to morphine in ethanol-habituated animals and man¹⁷, inhibition of ethanol withdrawal convulsions by morphine¹⁸, and attenuation of ethanol-induced narcosis by naloxone¹⁹ have all been demonstrated. Furthermore, the TIQ alkaloid, 3-carboxy-salsolinol (the L-dopa-acetaldehyde adduct) potentiates ethanol-induced narcosis²⁰ and morphine analgesia¹⁰ in mice, and salsolinol (the dopamine acetaldehyde adduct) interacts with the opiate receptor of the guinea pig ileum⁹. Our demonstration of inhibition of ethanol-induced withdrawal convulsions and blockade of ethanol-induced calcium depletion by naloxone provides further evidence for a functional relationship between opiates and ethanol.

We emphasise, however, that the results of these experiments and the others mentioned here, showing antagonism by naloxone of non-opiate drugs, must be viewed with caution. It is possible that naloxone, although considered to be a specific opiate receptor antagonist¹¹, has other actions so far unappreciated. The results would then simply reflect the action of naloxone at this alternate site(s) of action.

Nonetheless, the present state of knowledge supports our interpretation that ethanol and the opiates share some common biochemical mechanisms, and further research to delineate these mechanisms is warranted.

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Frog retinal ganglion cells show species differences in their optimal stimulus sizes

THE purpose of this study was to compare the optimum stimulus sizes of class 2 ganglion cells in *Rana pipiens* with those of *R. catesbeiana*. Several neurophysiological studies have suggested that activity in class 2 ganglion cells in the frog retina (convexity sensitive^{1–3}) serves as a trigger for prey catching^{1,4}. Field studies of the stomach contents of various species of frogs show that the prey selected by the bullfrog, *R. catesbeiana*, differs from that of the leopard frog, *R. pipiens*, in size. Bullfrog stomachs have contained (in addition to insects) crayfish, frogs, mice, and birds^{5,6}. Leopard frogs generally prey on various insects, spiders, and terrestrial snails^{7,8}. This study was undertaken to explore a possible difference in the electrophysiological optimal stimulus size for class 2 retinal ganglion cells of leopard frogs and bullfrogs which might be related to differences in prey size preference. Our data suggest that bullfrog ganglion cells have average optimal stimulus sizes significantly larger than those of leopard frogs.

The experiments described were conducted on adult frogs of northern North American origin. Leopard frogs ranged in body weight from 14.0 to 46.5 g, while bullfrogs weighed from 222 to 440 g. All frogs were acclimatised in the laboratory at 20–22 °C. for at least two weeks before use and were fed every two days. The animals were anaesthetised for the duration of the surgery with MS:222. The cranium was opened to expose the tectum. Paralysis was achieved with injection of neuromuscular blocking agents and xylocaine was infused into the incision site. MS:222 was then discontinued for the recording period. Frogs were dark-adapted for 1 h before recording began. Conventional electrophysiological techniques were used to record from optic nerve terminals in the tectum⁹. Platinum-plated, alloy-filled glass micropipettes of 3–5- μ m tip diameter¹⁰ were employed for the recordings. Electrical data were recorded on an A.M. tape recorder for later analysis. Spike trains from single neurons were resolved with a pulse height discriminator and the action potentials counted using a PDP-11 data acquisition computer system.

Visual stimuli consisted of black, round spots projected on to the back of a translucent tangent screen situated 24 cm from the frog's eye. Background illumination of the screen measured at this distance with a Weston Model 614 foot-candle meter

was 3–4 ft cd. The spots were produced photographically on a transparent film strip and ranged from 2.3° to 28° in diameter (i.e. the angle which the diameter of the spot subtended at the eye) in seven discrete sizes. Each size was presented twice, with 35 s allowed between presentations. A motor-drive moved the filmstrip through a projector so that the spots travelled horizontally across the screen at a constant, 7° s^{-1} angular velocity, through the centre of the excitatory receptive field (ERF) of each cell.

The topography of the retino-tectal projection onto the optic tectum was found to be similar in the bullfrog and leopard frog, and the same as described for other Ranid species^{11,12}. In both species, units were recorded from the mid-lateral region of the tectum which receives input from the central visual field to avoid disparities which might be caused by sampling from different fields. Class 2 cells were identified according to the following criteria: the cell responded best to small moving objects; it showed a sustained, movement-gated response to a stationary stimulus positioned in the ERF⁹; the sustained response had to be 'erasable', i.e. transient darkness would permanently extinguish the on-going response^{2,9}; and the cell showed a brief response to the general 'OFF' of light, but a weak, habituating response to the 'ON'. After the cell was identified as a class 2 type, the approximate extent of the ERF was mapped with a 2° spot. Then the response of the cell to each of the seven different stimulus sizes was determined. The computer was used to count individual spikes produced by the cell and also to measure the between-spike intervals. Spikes occurring after intervals longer than 50 ms were discarded and the response was then scored as the number of action potentials greater than or equal to 20 Hz. This frequency was chosen in order to eliminate background and low level activity. The cell's response was plotted against the logarithm of the area of each stimulus spot (Fig. 1). In order to determine the optimal stimulus size from the experimental data, we fitted a quadratic function to each cell's response profile (Fig. 1), using a computerised, stepwise, polynomial regression procedure. The goodness of the fit was tested for each case by an *F* test. All values of *F* so obtained were greater than 5.7 (d.f. 2/11) which is significant at better

than the 0.025 level (most values exceeded the 0.01 level). By differentiating the quadratic equation, we calculated the maximum of the curve which lies over the stimulus area that elicited the maximum response. From this area, we calculated the diameter of the optimum stimulus spot size in degrees for each cell, individually.

The results from 25 cells from 8 leopard frogs and 31 cells from 12 bullfrogs show that the optimum stimulus sizes of leopard frog class 2 cells average 5.22° (s.e. 0.3°), while the average for the bullfrogs is 7.77° (s.e. 0.21°). These values indicate that the bullfrog optimum stimulus requires a 50% larger diameter, or a 100% larger area than the optimum for leopard frogs. The null hypothesis that these two samples come from the same population may be rejected at $P < 0.001$ (Mann-Whitney *U* test). Our size optimum found in *R. pipiens*

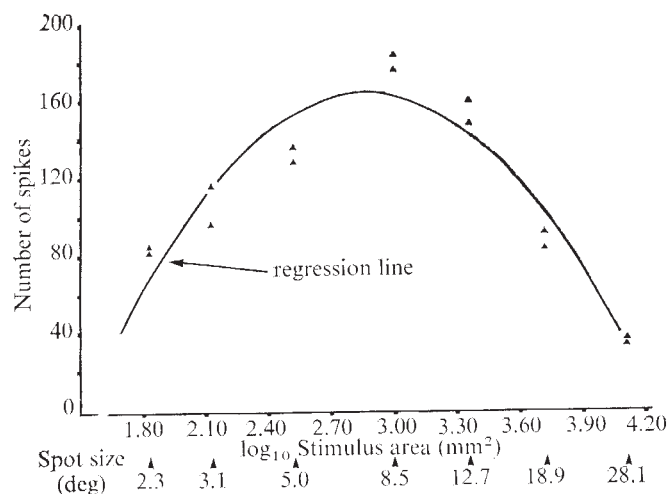


Fig. 1 Data obtained from one bullfrog class 2 ganglion cell. This figure demonstrates the technique used to determine the optimal stimulus size for the neuron. ▲, The response of the cell to the various spot sizes used. Each spot size was tested twice. The curve is the quadratic function fitted by a computerised polynomial regression procedure ($F = 46.4$). For a quadratic equation of the form $y = a + bx + cx^2$, the point of zero slope, $dy/dx = 0$, is given by $b + 2cx = 0$. This yields $x = -b/2c$ which predicts the maximum of the curve. In the case illustrated above, the maximum lies over the log stimulus area of 2.88, corresponding to a spot size of 7.4° or a stimulus area of 758.6 mm^2 . The equation of the regression line shown is $y = 544.5 + 490.9x - 85.3x^2$.

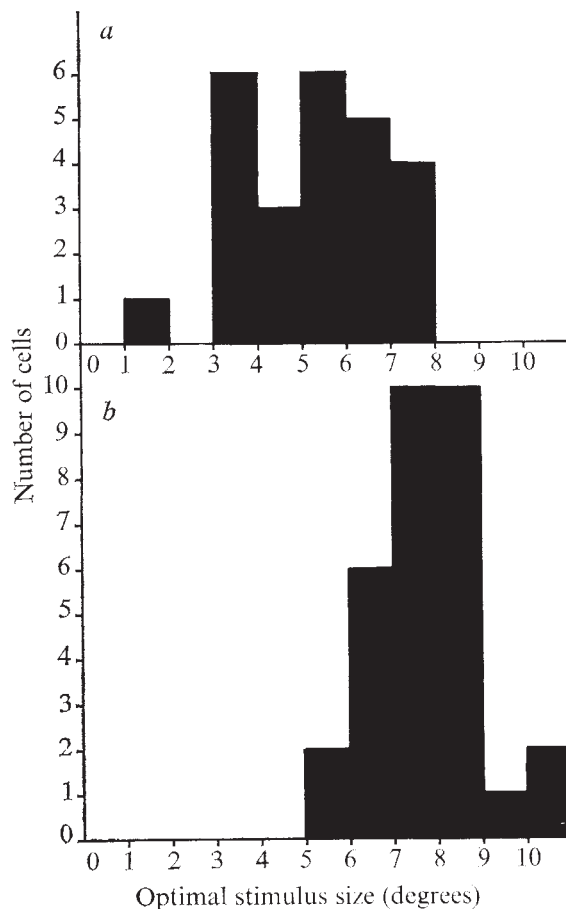


Fig. 2 Distribution of optimal stimulus sizes for 25 leopard frog (a) and 31 bullfrog (b) class 2 retinal ganglion cells determined by single unit recording of the responses of these cells to spots of different sizes moved across their receptive fields at a velocity of $7^\circ/\text{s}$. The average optimum stimulus size for the bullfrog cells was 7.77° , compared to 5.22° for leopard frog cells. Of bullfrog cells, 74% had size optima exceeding 7° , while 84% of leopard frog cells had a lower size optima.

is comparable to the 3° – 5° optimum found in *R. esculenta* class 2 cells by Butenandt and Grüsser¹³. This European frog is similar in size to the leopard frog. More interesting, perhaps, than the mean of the optimal stimulus sizes is the distribution of these sizes (Fig. 2). Although there is overlap of size optima between both species, bullfrogs exhibit many cells with larger optima. Of our sample, 74% of bullfrog cells responded best to objects larger than 7° , whereas 84% of leopard frog cells responded best to objects smaller than this size.

Two conceivable difficulties with the interpretation of our results deserve mention. First, we may not have sampled cells from comparable parts of the retina in both species of frog. Across many species of vertebrates, receptive field sizes of

ganglion cells increase from retinal centre to periphery¹⁴. Optimum stimulus size is roughly proportional to receptive field size in the frog retina¹³. If the optic axis of the bullfrog is different from that of the leopard frog, we could have sampled cells from non-analogous retinal regions, thereby introducing a possible discrepancy into our measurements. However, the similarity of the retinotectal projection between the two species which we have observed, and the similarity of their visual fields of view as revealed by perimetry¹⁵, lead us to doubt that a significant difference exists in the optic axis. Secondly, there is the possibility that the two species of frogs studied have had different refractive errors, resulting in varying degrees of retinal defocusing when both animals viewed the projection screen at the same 24 cm distance. We performed reflection retinoscopy on paralysed frogs and detected consistent, but small differences in the refractive errors of the two species. Leopard frogs were slightly myopic and bullfrogs were slightly hypermetropic. The errors measured however were less than 1.5 diopters and therefore were not considered to be large enough to defocus seriously the retinal image or to cause substantial disparities in retinal image size.

Technically, we note that the retinal image size of a given object at a constant viewing distance will be larger in the bullfrog eye than in the leopard frog eye, assuming that the refractive indices of cornea, lens, and vitreous are identical in both species. The linear extent of the image on the bullfrog retina will exceed that of the leopard frog in the same proportion as the ratio of the posterior focal lengths of their eyes. This disparity in size would lead one to predict that bullfrog retinal ganglion cells, if there were no differences in retinal circuitry, would have smaller optimal stimulus sizes than those of leopard frogs. Our observation, however, that in fact the reverse is the case, supports the existence of such a difference.

Behavioural experiments have indicated that, in addition to size cues, other factors are important for initiating prey catching in frogs. Olfactory input suggestive of prey can influence toads to choose larger than usual prey objects¹⁶. Motivation to feed may affect size preference¹⁷ as does satiety level¹⁸. The contrast of the object with the background as well as the season is significant¹⁹. 'Predator-like' visual stimuli excite neurons in the thalamus and pretectum which in turn inhibit tectal neurons, thereby blocking prey catching^{20, 21}. Single unit recording of retinal ganglion cells cannot elucidate all the complexities of prey catching that behavioural experiments have revealed (see, for example, Ingle²²). Prey catching does not follow activity in class 2 fibres in a one to one fashion. It is true, however, for the frog retina, that highly processed information about a visual stimulus is presented to the brain. We hypothesise that encoded in class 2 responses are size cues which may be one of several important triggers for prey catching. The differences in the size cues emitted by the retinae of frogs of different species may be the first step in producing a difference in prey size preference.

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Normal postimplantation development of mouse parthenogenetic embryos to the forelimb bud stage

PRE- AND POSTIMPLANTATION development of parthenogenetic embryos is of interest in analyses of the role of the male genome in early mammalian development, differentiation, and in haploid and diploid gene expression in embryogenesis. Diploid and haploid mouse parthenogenones evidently possess the capacity to form teratomas and give rise to differentiated tissues^{1,2} although normal organogenesis has not been observed to progress beyond the early somite stage *in utero*³. Birth of live parthenogenetic rabbits has been reported^{4–8}, and Beatty⁹ has estimated that 1 in 200 embryos induced under similar conditions can be expected to reach term. There is, however, some doubt about the validity of this early work since subsequent attempts to repeat it have failed to obtain development beyond the blastocyst stage^{10,11}. Mouse parthenogenones develop to the egg cylinder stage but only one 8-somite embryo has so far been obtained^{3,12}. We have examined the postimplantation development of diploid mouse parthenogenones and report that a high proportion progressed to somite embryos when blastocysts were transferred to pseudopregnant recipients. Recipients were ovariectomised following embryo transfer and their pregnant state maintained with exogenous hormones. Two apparently normal forelimb-bud stage embryos with a beating heart and yolk sac circulation were obtained. Other embryos isolated from the same recipient showed disorganised development, and a twin conceptus was obtained from a further recipient.

Cumulus masses from the oviducts of 8–12-week old (C57BL×CBA)F₁ hybrid mice were released at about 18 h after the HCG injection for superovulation into modified Krebs–Ringer bicarbonate embryo culture medium¹³ containing 4 mg ml⁻¹ bovine serum albumin but lacking calcium and magnesium salts (M.A.H.S. and M.H.K., in preparation). After 5–6 h adherent cumulus cells were removed with hyaluronidase, and the eggs examined to determine the activation frequency and types of parthenogenones induced (Table 1). Activated eggs were separated into their different types, transferred to normal embryo culture medium¹³ and retained in culture for a further 90 h. By this time 91 out of 178 of the presumptive diploid^{14,15} 2-pronuclear embryos had developed to the expanded blastocyst stage. Blastocysts were transferred to a single uterine horn of d 3 pseudo-pregnant recipients previously mated to proven sterile vasectomised males (day of finding vaginal plug=d 1 of pseudopregnancy). The recipients were bilaterally ovariectomised immediately after transfer of blastocysts and thereafter maintained on exogenous steroid hormones. After an initial hormone-free period of 2 d, recipients were maintained for 4 d on 1 mg progesterone daily per animal, during which time blastocysts presumably enter into quiescence.

Table 1 Types of parthogenones induced in HCG+18 h group in medium lacking Ca^{2+} and Mg^{2+}

Total no. of eggs examined	1 pronucleus + 2PB*	Activated eggs (%)		Immediate cleavage	Overall activation frequency (%)
		2 pronuclei without 2PB	1 pronucleus without 2PB		
731	58 (11.8)	243 (49.3)	27 (5.5)	165 (33.5)	67.4

*Second polar body.

Implantation was initiated by injecting 20 ng oestradiol along with progesterone for 3 d, and thereafter pregnancy maintained with 8 ng oestradiol and 1.6 mg progesterone daily per female.

Implantation began about 24 h after the initial injection of oestradiol, which is equivalent to about d 5 of pregnancy, and recipients were killed at intervals between the 6th and 11th day of "pregnancy". About 80% of the transferred

than electrical stimulation of eggs *in vivo*³ or hyaluronidase activation *in vitro*^{16,17}. The choice of the F_1 hybrid where one-cell eggs develop to the blastocyst stage *in vitro*^{15,16} is unusual since embryos from other strains do not normally progress beyond the 2-cell stage *in vitro*. The use of ovariectomised recipients may permit a better synchrony between blastocysts and the uterus before implantation commences. Blastocysts in this instance were allowed to

Table 2 Implantation rate and development of diploid (2 pronuclear) parthogenetic blastocysts in ovariectomised recipients given exogenous steroids

Day of "pregnancy" at autopsy	Total no. of recipients	Total blastocysts transferred	Total implantations	Total embryos	Approximate stage of development
6	1	3	3	1	} Early egg-cylinder
7	2	14	10	3	
8	1	13	10	4	Advanced egg-cylinder
10	3	15	12	2	4-8 somite embryos
11	2	9	8	5	25 somite embryos ($\times 2$)
					Disorganised embryos ($\times 2$)
					Twin conceptus, one with 10 somites

blastocysts implanted (Table 2), and all implantation sites up to d 9 were immediately fixed in Bouins solution and examined histologically. After d 8 of "pregnancy", embryos were dissected from the uterus and examined under a dissecting microscope. The two most advanced embryos obtained were at the forelimb-bud stage on the 11th d of "pregnancy", both had about 25 somites and were apparently healthy with beating hearts and a good yolk sac circulation at the time of isolation from the uterus. These embryos were later fixed for histology (Fig. 1a and b).

Several factors may account for the improved results reported here compared with the only other comprehensive study on postimplantation mouse parthenogenetic development^{3,12}. The mode of activation of eggs in $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free medium has not been used before and may be less traumatic

enter into quiescence before implantation was stimulated. An increase in cell number occurs as embryos enter into quiescence¹⁸ and would approximately double the number of cells in blastocysts before implantation.

Parthenogenetic blastocysts are similar to normal fertilised blastocysts when examined ultrastructurally¹⁹. Their cells can also differentiate into a variety of cell types in ectopic sites^{1,2}. In the present study 35% of implanted embryos developed into normal egg cylinders, and 25% developed to somite-stage embryos.

The present study indicates that non-fertilised parthenogenetic eggs are capable of apparently normal development to a very advanced state in the absence of any genetic or extragenetic contribution from sperm. Since a greater number of embryos develop normally after fertilisation, the

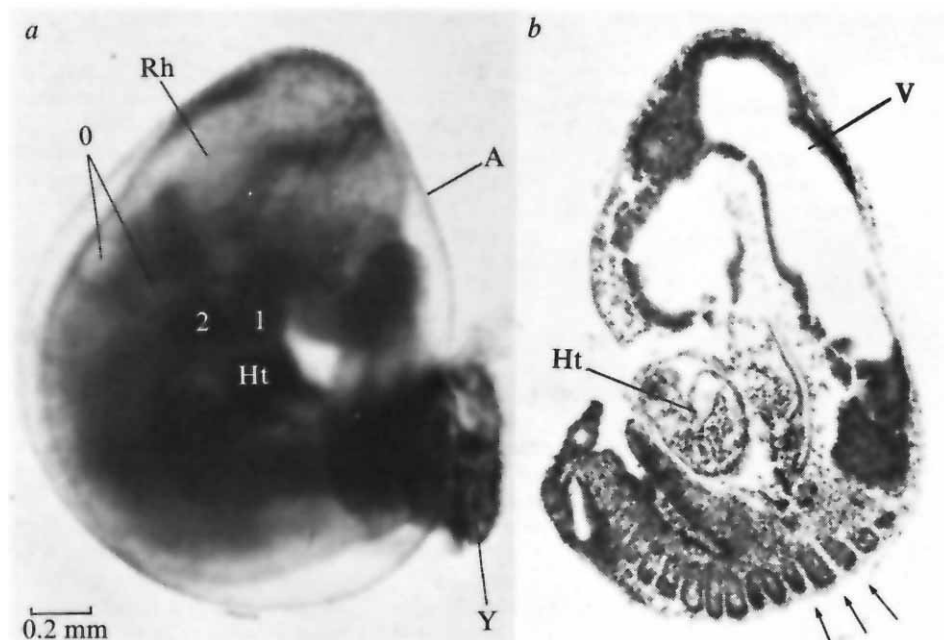


Fig. 1 a, Parthenogenetic mouse embryo with about 25 somites photographed within its amnion, unfixed preparation. b, Near sagittal section of embryo in a showing large number of well formed somites (arrowed). Embryo fixed in Bouins about 4 h after isolation from the uterus. Scale bar represents 0.2 mm; 1 and 2, first and second branchial bars; O, otic vesicle; Ht, heart; Rh, rhombencephalon with 4th ventricle; A, amnion; Y, yolk sac; V, 4th ventricle.

role of sperm is probably to prevent imbalance in the genetic constitution of the zygote. In the present study 60–70% of the embryos which successfully implanted died shortly thereafter, leaving about 25% to develop normally to somite embryos. In some embryos anomalous development may manifest itself in disorganisation of the embryo such that individual cells remain viable but are unable to form into tissues and organs. Survival of parthenogenones to term will depend on the extent of genetic imbalance in individual embryos which may affect development during critical stages of organogenesis.

Parthenogenetic embryos will be valuable to determine the developmental potential of genetically dissimilar embryos. The role of sperm in the activation of unfertilised eggs can be studied by morphological, genetical and biochemical comparison between fertilised and different types of parthenogenetic embryos. It may be possible to establish haploid and diploid cell lines from unfertilised parthenogenones. This would facilitate biochemical studies on cell surface constituents and their role in histotypic contact and cellular interactions. The disorganisation of some postimplantation parthenogenones probably reflects abnormalities in their cell surface properties which may be analysed by comparisons with the cells of normal embryos.

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Antigenic deletion and malignant enhancement induced in lymphoma cells by passage through X-irradiated hosts

WE report here a new system by which lymphoma cells are induced to delete strong, virus-associated membrane antigens and, as a result, considerably increase their capacity for transplantation and metastasis. This system of inducing antigenic changes in tumour cells is interesting because it involves X-irradiation of the hosts, which is used widely in the treatment of human cancer.

We used lymphoma cells which originated in W/Fu rats injected at birth with Gross leukaemia virus (GLV)¹. The cells were obtained either fresh from rat thymomas or from our established LT₁ lymphoma tissue culture line that permanently replicates GLV². As a corollary of reproducing the virus, both kinds of lymphoma cells express strong murine leukaemia virus (MuLV) surface antigens (Fig. 2)

and are consistently rejected when transplanted into normal, adult, syngeneic W/Fu rats. In rats given 300–350 R total body X irradiation, however, lymphoma cells transplanted within 24 h subcutaneously or intraperitoneally (Table 1) grew progressively at the site of the graft, spread to distant sites occasionally and eventually caused the death of the hosts. Lymphoma cells from such tumours growing in X-irradiated rats were then transplanted into normal, adult, syngeneic recipients, and in contrast to lymphoma cells of thymomas and of tissue cultures (controls, Table 1) were not rejected but grew into large tumours that metastasised widely (Fig. 1) and killed the recipients. Thereafter, such lymphoma cells became serially transplantable in all syngeneic, normal, adult rats, consistently resulting in voluminous tumours, metastases and death.

As described before^{1–2}, thymoma cells and LT₁ tissue culture cells show GLV C-type particles at the plasma membrane and exhibit positive membrane (MF) and cytoplasmic (CF) immunofluorescence when stained with anti-MuLV serum in indirect immunofluorescence. The membrane fluorescence is ring-like, around the entire cellular circumference and is apparent on almost all cells (Fig. 2b), usually brighter on the cells from the culture than on those from thymomas. Lymphoma cells from tumours growing in the X-irradiated rats, however, exhibited different patterns of fluorescence. Only 10–20% of cells showed the



Fig. 1 Normal adult W/Fu rat with subcutaneous transplant of MuLV- lymphoma cells. Massive metastases to kidneys (K), axillary (A), parathyroid (T), pararenal (R), para-aortic (O) and inguinal (I) lymph nodes.

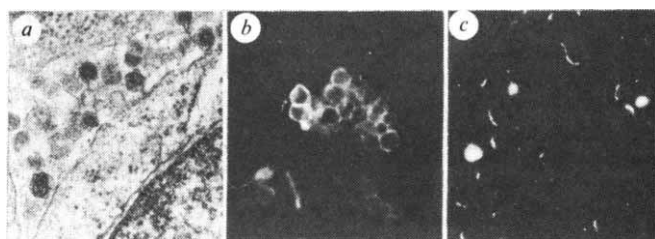


Fig. 2 *a*, MuLV+ lymphoma cell showing GLV particles at plasma membrane; *b*, MuLV+ lymphoma cell with ring-like membrane immunofluorescence; *c*, MuLV+ lymphoma cell with cap-like membrane immunofluorescence after modulation.

ring-like membrane fluorescence, while about 60% displayed capping of fluorescence (Fig. 2*a*) and 20% of the cells had become entirely negative. Tumours examined at longer intervals after transplantation in X-irradiated rats showed a progressive increase in the number of capped and negative cells with a proportionate decrease in the number of cells with positive ring-like fluorescence. Lymphoma cells of recipients that died less than 50 d after transplantation did not show loss of antigenicity as expressed by conversion from MuLV+ to MuLV- in the membrane immunofluorescence assay. Table 2 indicates that a minimum time interval was essential for the antigenic conversion to occur. As the tumours became serially transplantable to new generations of normal adult rats all lymphoma cells became permanently negative in both membrane and cytoplasmic fluorescence (Table 1).

Examined under the electron microscope, the transplantable lymphoma cells appeared devoid of both mature and immature virus particles. To investigate the apparent loss of MuLV antigens further, cytotoxicity assays were carried out using serum of rats that had rejected MuLV-positive lymphoma cells against MuLV-negative lymphoma cells as target cells and MuLV-positive lymphoma cells as controls. The results, expressed as percentages of killed cells, again confirmed the lack of MuLV antigenic expression on the transplantable lymphoma cells. As reported when antigenic conversion was induced by other means²⁻⁵, in our experiments the loss of surface MuLV antigens was consistently accompanied by increased malignancy of lymphoma cells expressed by serial transplantability and capacity for widespread metastases (Fig. 1).

The titres of serum anti-MuLV antibodies were determined in all recipient rats by cytoplasmic indirect immunofluorescence using standard LT₁ cells as previously described³. The results (Table 1) unexpectedly showed higher anti-MuLV antibody titres (up to 1:4,096) in the normal recipients grafted with MuLV- cells that grew into progressive tumours than in those grafted with MuLV+ cells that were rejected. This might be explained by assuming that a small amount of MuLV+ cells, insufficient to induce

tumour rejection but able to elicit antibody formation, was still present in the long-lasting, voluminous MuLV- tumours.

In our experiments, lymphoma cells acquired the capacity for transplantation and metastasis, consequent on the loss of their membrane viral antigens, which occurred during a passage through X-irradiated hosts. The new population of MuLV- cells may have emerged by immunoselection of nonantigenic, less vulnerable cells that were either pre-existent in the general population or appeared as a result of antigenic modulation. Defined as the phenotypic suppression of a surface antigen, occurring as a result of the cell's exposure to a specific antibody in the absence of complement⁵, antigenic modulation has been reported in relation to various virus-associated^{3,6-11} and histocompatibility¹¹⁻¹³ antigens. We have reported the antigenic deletion of MuLV antigens on lymphoma cells passed through rats that have been rendered specifically unresponsive to these antigens at birth³⁻⁵ as well as loss and recovery of MuLV antigens by *in vivo* and *in vitro* cultivation⁹. The importance of such phenomena lies in their possible relation to the mechanisms by which allografts and tumour cells can be unrecognised and escape immunological destruction¹⁴⁻¹⁵.

In the work reported here, we are inclined to believe that the deletion of membrane viral antigens is the result

Table 2 Antigenicity and transplantability of lymphoma cells in relation to time after irradiation

Time after* irradiation (d)	MIF†		Transplantation‡ T/TR	%
	+	-		
17-20	4	0	0/4	0
21-40	11	3	2/14	14
48-61	2	2	2/4	50
63-102	0	13	13/13	100

*Time interval between irradiation/transplantation and assays.

†Membrane immunofluorescence: no. of rats with + or - MIF.

‡T/TR = permanent tumours/transplanted tumours.

of antigenic modulation, based on evidence from immunofluorescence that the membrane alterations occurred stepwise (patching-capping deletion), as well as on evidence from preliminary experiments (to be reported later), showing that the antigenic changes were reversible *in vitro*.

The occurrence of cellular antigenic modulation after irradiation might be related to a special state of immune imbalance created by the effect of X-rays. T lymphocytes are more susceptible to radiation effects than B lymphocytes¹⁶⁻¹⁸ and one result of X-irradiation is the creation of a temporary imbalance between the two major compartments of lymphoid cells. It is also possible that B cells are increased in the general circulation, not only in relative values but also in absolute amounts in compensation for the depletion of T cells or as a result of T-suppressor-cell destruction.

Table 1 Serial subcutaneous transplantation of GLV lymphoma from X-irradiated rats

Transplant generation	Treatment	Tumours*	Metastases†	Antigens‡	Antibodies§
1	300-350R	7/9 (77)	6/9 (66)	+ - -	144(8-512)
2	None	18/21(85)	12/21(57)	- - -	684(32-4,096)
3	None	20/21(95)	20/21(95)	- - -	72(61-256)
4	None	18/20(90)	18/20(90)	- - -	25 (4-64)
5	None	14/14(100)	14/14(100)	- - -	
Controls	None	0/23 (0)	0/23 (0)	+ + +	83(32-128)

In parallel experiments GLV lymphoma cells were injected intraperitoneally in 24 irradiated and 14 non-irradiated control rats. Results were similar to those shown here.

*No. of rats with tumours at the site of subcutaneous injection/no. of rats transplanted. Percentages are given in parentheses.

†No. of rats with distant metastases/no. of rats transplanted. Percentages are given in parentheses.

‡Membrane GLV antigens in indirect immunofluorescence (each symbol represents 33% of rats).

§Titres of serum anti-MuLV antibodies determined by cytoplasmic indirect immunofluorescence on standard LT₁ tissue culture GLV-producing cells, as previously described³. Figures are means with the ranges in parentheses.

Antibody stimulation of tumour growth has been reported in T-cell depleted animals¹⁹, and an excess of antibody in irradiated, B-cell-stimulated, recipients might explain the immune pressures that induce antigenic modulation.

In man, postoperative irradiation is used and credited with prevention of recurrent and metastatic tumours. But in the light of modern concepts of the role of immune surveillance in the control of tumours, the effect of X irradiation, one of the strongest immunosuppressants, has been reviewed critically. Retrospective²⁰ and prospective²¹ studies of patients with breast cancer treated by radical mastectomy with or without postoperative irradiation showed marked increases in the number of lung and skin metastases in the former group, suggesting that radiotherapy, while effective in the control of local recurrences, might induce an increase in the number of distant metastases.

Whereas such adverse results are not entirely surprising in view of the immunodepressant effect of X irradiation, the mechanisms of its activity are not clear. Our experiments, demonstrating changes of lymphoma cells induced in X-irradiated hosts as a result of immunological imbalance, suggest a possible mechanism.

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Suppressor T-cell inactivation of a helper T-cell factor

THE ability of a subpopulation of T cells to inhibit both cellular and humoral responses is well documented^{1,2}. These regulatory or suppressor T cells have been produced by both antigen-specific and nonspecific stimuli, and both antigen-specific and nonspecific suppressive mechanisms have been suggested. In various studies T cells, B cells and macrophages have all been implicated as the primary target of the suppressor cells. We^{3,4} and others^{5,6} have reported

the nonspecific activation of both helper and suppressor murine T cells by concanavalin A (con A). The helper cell produces a nonantigen-specific mediator (NSM), of molecular weight 35,000, which can substitute for antigen-specific helper cells in the *in vitro* plaque-forming-cell (PFC) response of murine B cells to the antigen, sheep red blood cells (SRBC)^{3,4}. We report here the inhibition of this response by con A-activated suppressor cells and present evidence that the mode of action of the suppressors in this system involves the inactivation of the helper factor, NSM, and not a direct effect on the responding B cells.

We initially observed that in cultures containing B cells, SRBC and NSM the anti-SRBC PFC response was severely suppressed by the addition of small numbers of spleen cells which had been activated by 48 h of culture with Con A. Extensive titrations of both the helper factor and the suppressor cells indicated a competitive relationship between the two. The results of a typical experiment are shown in Fig. 1. B cells cultured alone with SRBC produced no anti-SRBC PFC. An increasing PFC response was obtained with the addition of increasing amounts of NSM until saturation was reached. The addition of various numbers of suppressor cells to these cultures generated a series of titration lines roughly parallel to the control titration, but shifted so that the amount of NSM necessary to achieve a given response increased with the number of suppressors.

An apparent competition between con A-activated helper and suppressor T cells was originally reported by Scavulli and Dutton⁷. Because we used the cell-free helper factor, NSM, the possibility of a direct interaction between the helper and suppressor T cells seemed to be eliminated as an explanation for the competitive effect. Our results suggested instead either that con A-activated suppressor cells can

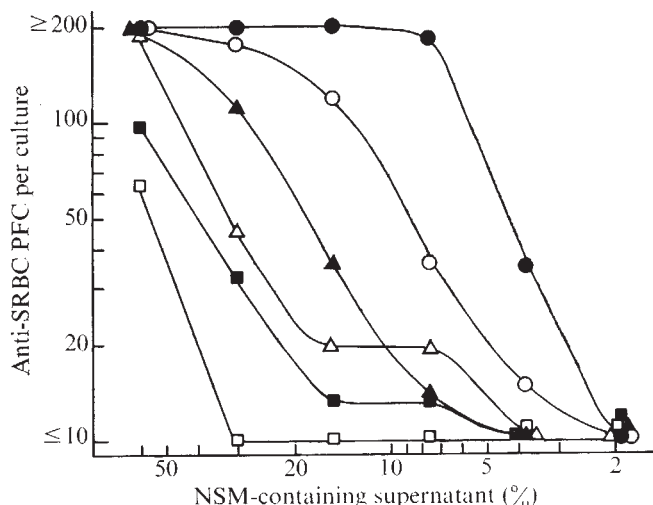


Fig. 1 Competition between NSM and suppressor cells. B cells were prepared from the spleens of BDF₁ mice (Jackson Laboratories) as previously described^{3,4} using both an *in vivo* treatment with anti-thymocyte serum (Microbiological Associates) and an *in vitro* treatment with specific anti-T-cell serum and complement. Microcultures were prepared by modifications^{3,4} of the methods of Mishell and Dutton¹⁶. Each culture well contained 2×10^5 B cells and 0.01% SRBC as the antigen in 0.1 ml of either medium or medium containing the various proportions indicated of an NSM-containing, 24-h supernatant from normal spleen cells cultured at 10^7 per ml with con A ($4 \mu\text{g ml}^{-1}$)^{3,4}. Residual con A had been removed from the supernatant by absorption with Sephadex G-200^{3,4}. In addition microcultures contained either 0 (●), 7.5×10^2 (○), 1.5×10^3 (▲), 3.0×10^3 (△), 6.0×10^3 (■), or 1.2×10^4 (□) suppressor cells produced by culturing normal spleen cells for 48 h at 10^7 per ml with con A ($4 \mu\text{g ml}^{-1}$). After 4 d individual cultures were assayed for anti-SRBC PFC. Each point represents the average of the responses assayed in 12 identical cultures. In the absence of NSM all responses were less than 10 PFC per culture.

interact directly with NSM, or that they compete with NSM in their effects on B cells.

The possibility of a direct suppressive effect on B cells was eliminated in a second group of experiments. Several workers⁸⁻¹² had shown that B cells responding to SRBC were induced by the antigen to undergo at least one round of division in the absence of helper factors. Helper cells or factors were important in the response only after 24–48 h, when their addition was essential for the continued proliferation and differentiation of the responding B-cell clones to the point of antibody secretion. We looked at the effect of suppressor cells on this response when NSM was not added to cultures until 24 h after the initiation of the response by antigen (Fig. 2). The responses of control cultures were similar whether NSM was added at zero time or at 24 h. The results, however, clearly showed that the presence of suppressor cells throughout the culture period was not a sufficient condition for suppression, for suppression was not seen if the addition of NSM was delayed 24 h. This result eliminated the possibility of a direct inhibitory effect of the suppressor cells on B cells.

There are several possible explanations for the requirement that NSM must be present from the beginning of the response in order for suppression to be observed. The possibility that we explored further was that, because B cells do not develop the requirement for NSM until at least 24 h into the response, the presence of suppressor cells and NSM together during this period might lead to NSM inactivation. When added at 24 h, the NSM might interact with the then receptive B cells before its inactivation by suppressor cells.

To test the possibility that suppressor cells act directly on NSM we preincubated concentrated aliquots of NSM for 24 h alone or with various concentrations of suppressor cells. The cells were then removed by centrifugation, and the supernatants tested for NSM activity on B cells which had been cultured for 24 h with SRBC. As Fig. 3 shows, the NSM preparation preincubated alone produced a normal

anti-SRBC response, whereas preincubation with increasing numbers of suppressor cells yielded successively less active supernatants. It should be noted that the ratios of suppressor cells to NSM in the preincubation step were similar to those which produced suppression in the experiments reported in Figs 1 and 2. These results indicated that the full activity of the suppressor cells could be accounted for by the direct elimination of NSM activity.

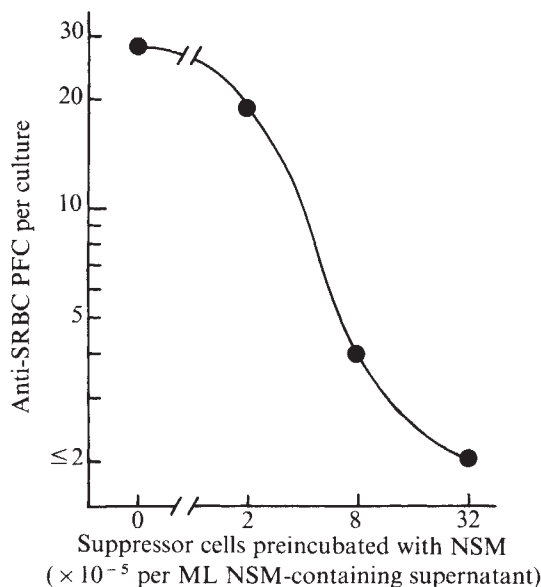


Fig. 3 Inactivation of NSM by preincubation with suppressor cells. Neat NSM-containing supernatant, prepared as in Fig. 1 was preincubated for 24 h at 37 °C either alone or with various concentrations of suppressor cells, prepared as in Fig. 1. B-cell cultures containing SRBC were prepared as in Fig. 1. After 24 h the various preincubated supernatants from which cells had been removed by centrifugation were added to the B-cell cultures to a final concentration of 10%. After an additional 3 d all cultures were assayed for anti-SRBC PFC. The results of four similar experiments were combined. Each point represents the average response of from 36 to 48 individual cultures. In the absence of NSM the response was less than 2 PFC per culture.

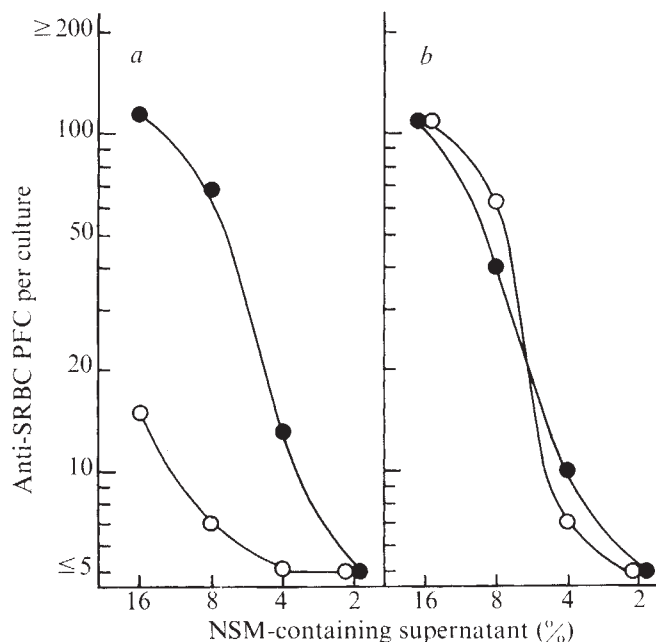


Fig. 2 Loss of suppression with the delayed addition of NSM. NSM-containing supernatant, suppressor cells and B-cell cultures containing SRBC were prepared as in Fig. 1. *a*, A group of B-cell cultures received various proportions of NSM supernatant at time zero (●). A second identical group received in addition 2×10^3 suppressor cells at time zero (○). *b*, Same as (*a*) but the addition of NSM was delayed until 24 h. All cultures were assayed for anti-SRBC PFC on day 4. The results are pooled from two identical experiments. Each point is the average response of 24 identical cultures. In the absence of NSM all responses were less than 5 PFC per culture.

Several additional control experiments were performed. For example, the cell responsible for the NSM inactivation was shown to be eliminated by treatment with anti-T serum and complement whether before or after con A activation (data not shown). Typical results of a second control experiment are shown in Fig. 4. As expected, NSM preincubated with excess suppressor cells lost all activity. Preincubation of NSM with the same number of normal spleen cells, however, resulted in no loss of NSM activity, demonstrating the need for con A activation to produce the suppressor cells. In addition, the loss of NSM activity was not due to its masking by an inhibitory factor produced by the suppressor cells or to the conversion of NSM to an inhibitory factor, for either medium or NSM preincubated with suppressor cells had no effect on the activity of fresh NSM. These latter results also suggest that the loss of NSM activity was not through the action of a soluble degradative enzyme, but rather required direct interaction of the NSM with the suppressor cells. We are attempting to distinguish whether this interaction resulted simply in the stoichiometric absorption of NSM by a suppressor cell receptor or in its destruction at the cell surface.

In summary, our results indicate that the action of con A-activated suppressor cells on the response of B cells to the antigen, SRBC, and the helper factor, NSM, is through the inactivation of the NSM rather than a direct effect on B cells. They suggest that one mode of action of regulatory T cells is the control of the level of immunologically active mediators.

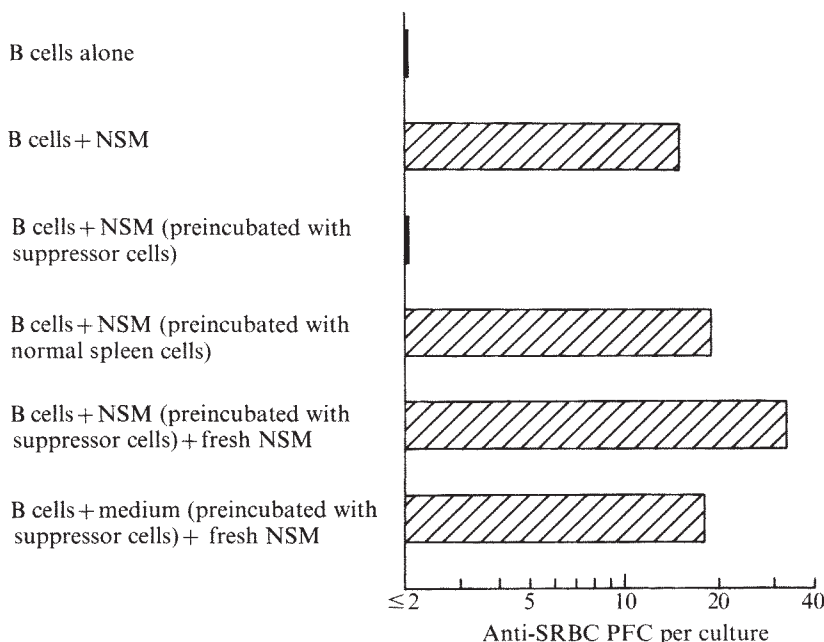


Fig. 4 Need for con A activation of the suppressor cells and the lack of the production of a soluble suppressive activity. NSM-containing supernatant, prepared as in Fig. 1, was preincubated for 24 h at 37 °C either alone or with 8×10^3 suppressor cells per ml or with 8×10^3 normal spleen cells per ml. In addition normal medium was preincubated with 8×10^3 suppressor cells per ml in the same conditions. B-cell cultures containing SRBC were prepared as in Fig. 1. After 24 h the various supernatants prepared as above were added to the B-cell cultures to a final concentration of 10%. Some cultures received in addition fresh NSM supernatant to final concentration of 10%. After a further 3 d all cultures were assayed for anti-SRBC PFC. Each bar represents the average response of twelve identical cultures.

Our results are somewhat at odds with those of Pierce *et al.*^{13,14}, who have proposed that the activity of con A-activated suppressor cells is accounted for by the production of a factor, indistinguishable from macrophage migration inhibitory factor, whose mode of action is to cause normal macrophages to become suppressive. Perhaps macrophages stimulated in this manner also develop the ability to inactivate helper factors. Our results also raise a question concerning the clearly demonstrated ability of con A-activated suppressor cells to inhibit responses to thymus-independent antigens^{3,4,15}. Because these responses presumably do not involve helper factors, the mechanism of suppression is unclear. A direct interaction between suppressor cells and this special type of antigen is an intriguing possibility; however, alternative suppressive mechanisms involving direct effects on B cells are also possible.

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Androgen-modified expression compared with Y linkage of male specific antigen

SEVERAL recent results have led to a widely held view that the male specific antigen (MSA) is coded for by a structural gene on the Y chromosome, and because of this it is frequently termed H-Y (refs 1 and 2). Although a good case has been put forward for this conclusion, there are some data that conflict with this view and some arguments against the notion. These are presented here as a reminder that, as is so often the case with biological experiments, the Scottish verdict “not proven” holds.

Evidence in favour of a Y-linked structural locus for the MSA has led to a hypothesis that the Y-linked gene(s) required for testis development and the MSA structural locus are one and the same^{3,4}. It is theorised that MSA acts as a partially diffusible hormone able to induce male gonad formation in coated XX cells and evidence for this mechanism has been presented for bovine freemartins⁵. But strong evidence has been presented against Y linkage of the MSA⁶. This is the observation that XX, *Sxr* (sex-reversed) males have MSA. In spite of cytogenetic studies involving various new staining techniques, there is no evidence for translocation Y material in XX, *Sxr* males^{7,8} and the presence of MSA in XX, *Sxr* males is incompatible with Y linkage of the structural gene for MSA. Evidence for the presence of MSA in phenotypically female, XY, *Tfm* mice has been presented as evidence that the MSA is not a concomitant of maleness *per se* and, therefore, must be Y-linked. An alternative hypothesis states that the structural locus for the MSA is autosomal, that its expression is triggered by a male determinant normally controlled by a portion of the Y chromosome, and that apparent Y-linked allelic differences in the MSA are the reflection of altered levels of this Y-chromosome factor, due to allelic forms of the Y-chromosomal locus. *Sxr* could be a constitutive mutant of the autosomal locus normally induced by the Y-chromosome factor. According to this hypothesis, the evidence that MSA is present in phenotypically female *Tfm/Y* mice⁹, which have high circulating levels of testosterone, provides the limitation that, if the

Table 1 Summary of Y-linked 'fertility' data

Characteristic	Strains compared	Evidence for Y linkage	Ref.
Mean sperm dimensions	Strains selected for large virus small body size	Paternal cytoplasmic effect or Y-linked effect	45
% Morphologically abnormal sperm	C57B1 (high) against CBA (low)	Portion of effect Y-linked	46
% Morphologically abnormal sperm	KE (high) against KP (low)	Portion of effect Y-linked	47
% Morphologically abnormal sperm (with fertility inversely correlated)	KE (high) against CBA (low)	Portion of effect Y-linked	48
Male behavioural differences	PHH against PHL	Y-linkage accounts for all the difference	49
Male aggression	DBA/1/Bg against CBA/FaCam (low)	Y-linkage or maternal effect	50
Adult testes size	SF (high) against CBA/FaCam (low)	41 % Explained by Y linkage	51

MSA is mediated by testosterone, the hormone does not act through a target organ mechanism limited to males (the cytosol testosterone receptor thought to be altered in *Tfm*^{10,11} is present in females as well as males^{3,12}). This mechanism would already seem unlikely because of the presence of MSA on epidermal cells, where it is detected by skin grafting, and other tissues which are not primary testosterone targets.

Two other aspects of research on the MSA may be responsible for part of the confusion. First, the work on MSA has been performed by skin grafting; only recently has serological detection of the MSA been possible¹³. The antigen detected with mouse sera cross reacts with cells from other mammals studied, including man¹⁴, and even amphibia and birds, where the female heterogametic sex has the antigen¹⁵. Grafting techniques, however, have not established a MSA in man^{16,17} or amphibia¹⁸ but the lack of inbred strains in these species may be the problem, that is, it is not possible to make male-to-female grafts between individuals with otherwise identical genotypes. Immunological cross reactions between such widely divergent species are likely to be due to something other than a protein, for example, a carbohydrate or a steroid, or a protein whose function is extremely stable in evolution, such as histone IV. If one is to argue that the evolutionary conservation of the MSA reflect a conserved function, there is the problem that its function must be related to female sex determination in WW, WZ species. If the MSA is a protein coded for by the heterogametic sex chromosome, it might be simpler to postulate that it is a protein involved in the synthesis or packaging of the unique, heterogametic tandemly repeated DNA¹⁹. Second, differences in the MSA detected by skin grafting may not reflect different alleles but different loci, that is, there could be a MSA with a Y-linked structural gene and other MSA(s) which are autosome-linked and androgen-dependent.

The original evidence that the MSA locus was on the Y chromosome was of two sorts. First, teratoma lines maintained by *in vivo* passage have a tendency to lose the Y chromosome and apparently lose the MSA concurrently²⁰. But the reported loss of the MSA in teratomas which had lost the Y chromosome depended on very few grafts; some Y-containing cells persisted in the accepted grafts; and the work was not confirmed²¹. Second, there are various strengths of the MSA, originally detected by skin grafting²²⁻²⁴ and possibly found serologically¹³, which are Y-linked. Other workers, however, have not been able to reproduce some of the results suggesting alternative forms of a Y-linked antigen²⁵⁻²⁶. More recently, a dose relationship between the number of Y chromosomes and the amount of serologically detected MSA in humans has been used to argue for Y-linkage of the MSA²⁷. Studies on humans have also shown MSA in XX males with no detectable XY cell line²⁸, a situation analogous to *Sxr*, XX mice. Thus, the data are also highly compatible with the interpretation that MSA is a concomitant of maleness which can occur occasionally in the absence of a Y chromosome, for example, polled in goats²⁹, as femaleness

can occasionally occur in the presence of a Y chromosome³⁰.

There is a variety of evidence to suggest that the MSA depends on androgens for its expression. Neonatal orchiectomy of C57B1/6 males leads to prolonged, or even permanent, survival of the adults' skin on females of the same strain^{31,32}, while neonatal male skin grafts allowed to develop after being transplanted to adult females do not reach normal adult male skin immunogenicity for secondary female recipients until after more than 100 d³³. Furthermore, female responsiveness to male skin grafts is specifically depressed by testosterone injections started at birth³⁴, while skin from males pretreated with a non-functional analogue of testosterone, cyproterone acetate, has prolonged survival on females³⁵. Thus, expression of the MSA is not complete in these situations, in spite of the presence of the Y chromosome. Testosterone, however, can have inhibitory effects on the immune response³⁶ and this could explain effects on female responsiveness.

The observation that MSA expression can be modified by *H-2* region-linked genes³⁷, which is also controversial²⁴, could be explained by a previously described *H-2*-linked effect on testosterone levels^{38,39}. (The effect of *H-2* on MSA expression seems to be separate from the role of *H-2* in the specification of the MSA target cell complex for cell-mediated cytotoxicity⁴⁰, a role *H-2* antigens also play with hapten or virus-induced cell surface antigens⁴¹⁻⁴³.) It should be added that the anti-androgenic steroid also reduced expression of *H-3* and *H-2* antigens, as measured by the survival of skin grafts from treated males transplanted across those barriers⁴⁴. The latter result suggests that androgens could easily play an important role in the expression of antigens not linked to the Y chromosome, including the MSA.

There are various Y-linked differences among males of different inbred strains, and most of these putative Y-linked phenotypic differences could be secondary to levels of androgen (Table 1). The difference which should be most clearly related to androgen levels is that of testes size; 41% of the difference between CBA/FaCa and SF strains was Y-linked⁵¹. Thus, if the original data suggesting Y-linked alleles for the MSA are correct, these may be explicable as secondary effects of Y-linked differences in androgen levels. The Y chromosome "switch" for male-determination is postulated to have several alternative levels of expression. The apparent double dose of Y antigen in human males with two Y chromosomes²⁷ is also compatible with this hypothesis.

If the MSA is actually controlled by an autosomal locus but modified by androgen activity (with the latter possibly showing allelic variation controlled by the Y chromosome), the presence of MSA in phenotypically female, *Tfm*, XY mice⁹ argues that the action of androgens on the MSA is not through a mechanism limited to testosterone target organs of males. Testosterone seems to have important functions in female reproduction⁵² and to be involved in protein synthesis in the female liver⁵³. The latter function of androgen in females is mediated by microsomal membrane testosterone receptors⁵⁴ which are under hypothalamic control⁵⁵. There are other examples of steroid hormone

function which are not mediated by cytosol receptor-nuclear translocation mechanisms. There is evidence that oestrogen⁵⁶ and glucocorticoids⁵⁷⁻⁵⁸ have direct effects on intracellular cyclic AMP levels while oestrogen releases histamine from the rat uterus⁵⁹ and progesterone mediates effects on frog oocyte differentiation which involve an effect on the outer surface of the cell membrane, without a requirement for entrance of the hormone into the cell⁶⁰. Thus there are androgen responses which are not male, target-organ-limited, but if they are responsible for MSA expression they should not all be shared by the female. If they were, MSA should not be detectable because the antigen should be produced in the females and they would be tolerant. Rather, it is assumed that various androgen functions, some of the target organ type and some mediated by other pathways but male-limited, are "turned on" in the male by the Y-linked trigger which may have a primary effect on one autosomal locus such as the *Sxr* locus. Then it would be possible that the *Sxr* locus product is MSA⁶, while its expression would normally be controlled by a Y chromosome.

There are two major arguments against this alternative hypothesis. First, there is the reported detection of antigen cross reacting with murine MSA in females of birds and reptiles where the female is the heterogametic sex¹⁵. There is also evidence that female-to-male grafts are rejected in chickens⁶¹. But this creates a theoretical dilemma: if the antigen is a protein then it has been uniquely stable in evolution when its function has changed to become female determinative while if it is not a protein, the argument that Y-linked allelic forms demonstrate a structural locus on the Y chromosome is much more tenuous. Second, there is the apparent finding of MSA on putative XY mouse embryos at the eight-cell stage⁶². This cannot be explained by secondary effects of maleness but needs further confirmation: embryos were classed as "dead" if only one cell was lysed and in no case were more than five cells lysed. The 50% "death" of eight-cell embryos can be explained just as readily by assuming that all the embryonic cells are equally sensitive to antibody plus complement. If the probability that any given cell will be lysed is only 0.083, then 50% of the embryos will remain unaffected, while the remainder should have one or more lysed cells, the number of cells lysed per eight-cell embryo following a binominal distribution. Such a distribution predicts the presence of very few embryos with four or more dead cells. Although the actual data have not been presented, larger than expected numbers of embryos with four or more dead cells might simply reflect some degree of co-operativity, with the death of one cell rendering the adjacent cells more susceptible to lysis.

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Specific binding of a synthetic oligodeoxyribonucleotide to yeast cytochrome *c* mRNA

THE investigation of specific messenger RNAs in terms of sequence analysis and of the control of their synthesis, processing, translation and degradation is one of the major interests of modern molecular biology. These studies have been possible so far in only the few cases where a cell type is known to synthesise predominantly one species of protein. Since most mRNAs are chemically similar, differing only in size and sequence, it has been difficult to purify them by conventional methods. Given the amino acid sequence of a protein, one can use the genetic code to deduce a sequence of nucleotides in a specific region of the mRNA and then synthesise an oligodeoxyribonucleotide complementary to that region¹⁻⁴. We have chosen to investigate the yeast iso-1 cytochrome *c* mRNA as a model system for the use of synthetic oligonucleotides as specific probes of eucaryotic mRNA structure and function. The genetics of yeast iso-1 cytochrome *c* has been studied in great detail by Sherman, Stewart and co-workers^{5,6}, who have determined the sequence of 44 nucleotides of the mRNA from the analysis of altered cytochromes produced by a series of double frameshift mutants. In addition, a number of well defined deletion mutants are available within this region. The pentadecadeoxyribonucleotide (15-mer) that we decided to synthesise, 5'-d(A-G-C-A-C-C-T-T-T-C-T-T-A-G-C)3' is complementary to nucleotides 25-39 of the cytochrome *c* mRNA (Fig. 1). This sequence was chosen because it is relatively easy to synthesise, should form a very stable hybrid with mRNA, and is close to the 5' end of the coding region of the mRNA. A known deletion mutation covers this region, yet allows the production of a functional cytochrome *c* in normal amounts^{5,6}, and thus provides an excellent control for hybridisation experiments. We have been able to show that the

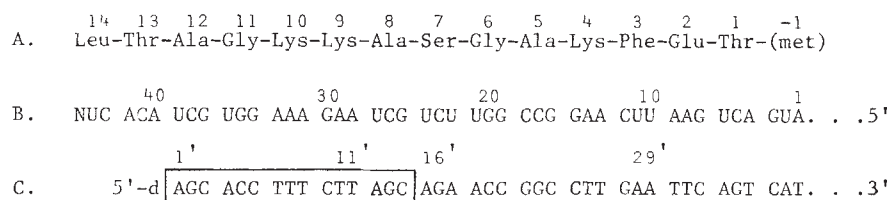


Fig. 1 Amino acid sequence of yeast iso-1 cytochrome *c* (a), and nucleotide sequence of its mRNA (b), with the complementary DNA sequence c. The boxed-in area represents the synthetic 15-mer.

synthetic 15-mer does indeed bind specifically to the expected region of the mRNA, and therefore should be useful as a specific probe for the study of this mRNA.

The chemical synthesis of the pentadecanucleotide was by the modified phosphotriester method of Narang and co-workers^{7,8}. Recent developments in this approach to synthesis, such as the introduction of new reagents for phosphorylation and condensation, have greatly reduced the time required to synthesise oligonucleotides 12–21 nucleotides 3 long. Our synthesis proceeded in three stages. First, completely protected mononucleotide 3' phosphates were prepared according to the procedures of Narang and co-workers^{8,10} except that the methylbutyryl group was used for the protection of the amino group of deoxyguanosine¹¹, and the 3' OH of the primer was protected by acetylation¹². Secondly, the monomers were condensed into di- and tri-nucleotides as described earlier^{8,10}. Finally, the five trinucleotides were assembled using mesitylenesulfonyltetrazole as a condensing reagent^{13,14}. The final product was deblocked and the 15-mer purified on a Sephadex G-50 column followed by chromatography on a DEAE-cellulose column in 7 M urea with a final yield of 10 μ mol.

We have confirmed the sequence of the synthetic oligonucleotide by direct sequence analysis (Fig. 2). The 15-mer was labelled with ³²P at the 5'-end with polynucleotide kinase¹⁵, or at the 3'-end with terminal transferase¹⁶. The labelled material was partially digested with venom or spleen phosphodiesterase, and mapped by electrophoresis on Cellogel strips followed by homochromatography^{15,17,18}. Each map consists of a series of spots which represent the products of partial digestion of the starting material. The mobility shift of each successive spot is characteristic of the added nucleotide¹⁹. In all cases the mobility

shifts are as expected. Furthermore, 5'-end analysis shows that >95% of the 5'-nucleotide is A and the 3' nucleotide is C.

Hybridization of the 15-mer to the cytochrome *c* mRNA can be detected by incubating bulk mRNA with an excess of the ³²P-labelled oligonucleotide under the appropriate conditions, and then separating the free from the bound 15-mer by chromatography on Sephadex G-75 or by electrophoresis in a 5% polyacrylamide gel. Both methods gave the same results but the latter method is most suitable for the simultaneous analysis of a large number of samples. We found that most of the binding to mRNA (>80%) from derepressed wild type yeast (grown

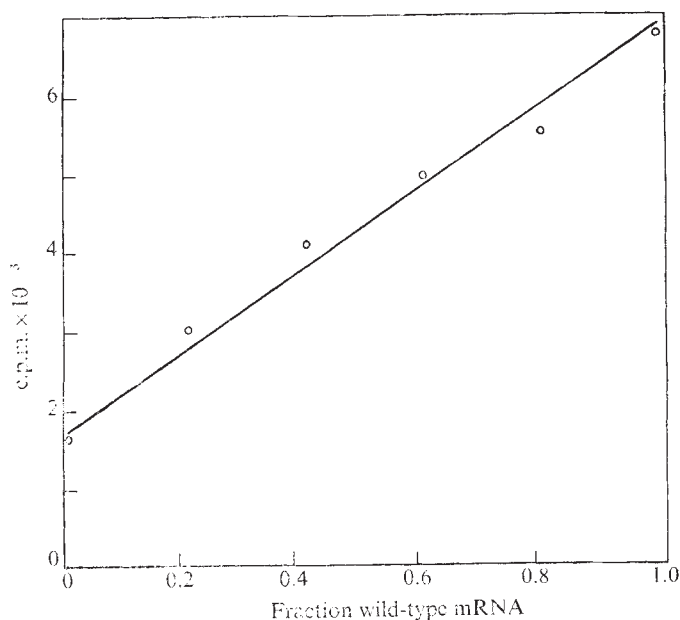


Fig. 3 Specific hybridisation between synthetic (³²P)15-mer and yeast cytochrome *c* mRNA. Total RNA was isolated by breaking chilled yeast cells in a Braun homogeniser in the presence of phenol, followed by centrifugation and ethanol precipitation of the aqueous layer. Electrophoretic analysis of this RNA showed very sharp ribosomal RNA bands with no evidence of any degradation products. The mRNA fraction was isolated from the total RNA on poly-U Sepharose column². 1.0 pmol of (³²P)15-mer and 50 μ g of mRNA were dissolved in 50 μ l of H-buffer (0.1 M NaCl, 0.01 M Tris-Cl, pH 7.5, 0.001 M EDTA, 0.05% SDS). The amount of mRNA from derepressed wild-type cells (strain D311-3A, *a*, *his1*, *trp2*, *lys2*) was varied from 0 to 50 μ g, with each sample made up to 50 μ g total with mRNA from derepressed deletion mutant cells (strain B2185, *aCYC-1-183-AD*, *his1*, *trp2*, *lys2*). Strains of yeast were a gift of Dr Fred Sherman. The reaction mixtures were incubated at 37 °C for 18 h, at which time 10 μ l of loading dye (60% sucrose, 0.2% bromophenol blue) was added to each sample. Electrophoresis was carried out for 3 h at 3 V/cm on a 5% polyacrylamide gel (5% cross-linking with bis-acrylamide). All mRNA and bound primer was contained in an area within 0.5 cm from the origin, which was cut out and counted. Approximately 0.1–1.0% of the input labelled primer was bound to mRNA.

with aeration on ethanol) is due to specific hybridisation to iso-1 cytochrome *c* mRNA. In control experiments, the binding of 15-mer to mRNA from either repressed wild type cells (grown under nitrogen with high glucose), or from derepressed cells which carry a deletion of the complementary region, is reduced to less than 20% of the binding to mRNA from derepressed wild type cells. The amount of 15-mer bound to wild-type mRNA is in direct proportion to the amount of mRNA present (data not shown). When the amount of mRNA in a hybridisation reaction

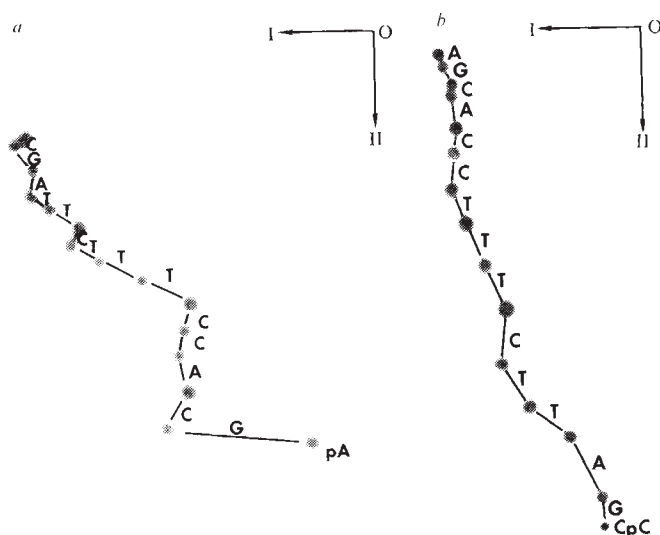


Fig. 2 a, Two-dimensional homochromatography of a partial venom phosphodiesterase digest of the synthetic pentadecanucleotide d(³²pA-G-C-A-C-C-T-T-T-C-T-T-A-G-C). Dimension I, electrophoresis on cellulose acetate strip (Cellogel, Reeve-Angel), pH 3.5 at 2000 V for 40 min. Dimension II, homochromatography on DEAE-cellulose plate (20 × 20 cm), with Homo-mix IV¹⁵. b, Two-dimensional homochromatography of a partial spleen phosphodiesterase digest of the 16-mer d(A-G-C-A-C-C-T-T-T-C-T-T-A-G-C)³²pC produced by transferase labelling with (α -³²P)CTP of synthetic 15-mer. Dimensions are as in Fig. 2a except that homochromatography was done on a 20 × 40-cm plate with Homo-mix III¹⁵. The migration of the spots is less in the electrophoresis dimension because there is an -OH group at both the 5'-end and the 3'-end of the oligonucleotides.

is made up to a constant amount with mRNA from deletion mutant cells, the amount of 15-mer bound increases in direct proportion to the amount of mRNA from wild type cells (Fig. 3). Therefore, the 15-mer is specifically hybridizing to the cytochrome *c* mRNA.

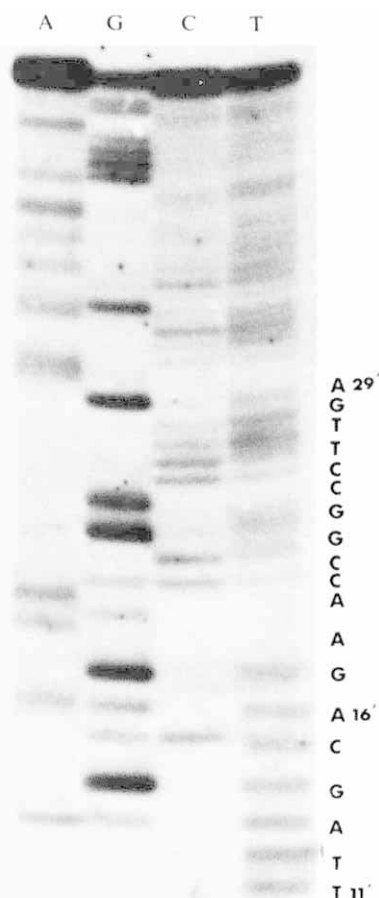


Fig. 4 Enzymatic extension of 15-mer primer hybridised to yeast cytochrome *c* mRNA. 5 mg of mRNA was incubated with 500 pmol of 5'-(³²P)15-mer (1000 mCi μmol⁻¹) in 5.0 ml of H-buffer at 37 °C for 44 h. The primer-mRNA hybrid (1.1 × 10⁶ c.p.m.) was separated from free primer on a Sephadex G-75 superfine column (2.4 × 35 cm) in 0.1 M NaCl, 0.01 M Tris-Cl pH 7.5. The 15-mer primer was extended by the incorporation of unlabelled dNTPs as follows¹⁹. The solution containing the primer-mRNA hybrid (11.0 ml) was adjusted to a final concentration of 25 mM HEPES buffer (pH 7.5), 1 mM MnCl₂, and 5 μM of all four dNTPs. *E. coli* DNA polymerase I (100 units) was added and the reaction allowed to proceed at 1.5 °C for 4 h. The NaCl concentration was adjusted to 0.3 M and 2 vol. of ethanol were added. The mixture was filtered to -20 °C for 30 min and centrifuged at 10,000 r.p.m. in an HB-4 rotor for 20 min. The pellet, which contained 99% of the radioactivity, was dissolved in 0.3 ml of 0.3 N NaOH and incubated at 37 °C for 16 h to hydrolyse the RNA. To fractionate the labelled extended primer, the sample was loaded on a 20 × 20 × 0.3 cm polyacrylamide gel (10% acrylamide with 5% cross-linking, 7 M urea, 50 mM Tris-borate, pH 8.3) and electrophoresed at 400 V until the bromophenol blue had migrated 37 cm. The gel was then autoradiographed, the labelled bands eluted, concentrated on a DEAE-cellulose column, eluted with 2 M NaCl, and ethanol precipitated. The products of the primer extension reaction were sequenced by the chemical method of Gilbert and Maxam²⁰. A-specific cleavage is obtained by partial purine methylation with dimethylsulphate, preferential depurination in dilute HCl, followed by chain cleavage with alkali. G-specific cleavage is obtained by partial methylation as above, followed by chain cleavage with piperidine. C- and T-specific digestions are obtained by partial hydrazinolysis (in the presence of 1 M NaCl for a C-specific reaction) followed by piperidine treatment to break the chain. The products of these four base specific partial digestions are separated on four lanes of a polyacrylamide slab gel (20% with 3% crosslinking, 7 M urea, 50 mM Tris-borate, pH 8.3). The autoradiograph of such a gel is shown above. The sequence is deduced from the presence of specific bands on each of the four lanes.

We have sequenced a short region adjacent to the binding site of the 15-mer as further proof that the 15-mer binds to the expected region of the yeast cytochrome *c* mRNA. DNA polymerase I will extend a DNA primer on a RNA template¹⁹ in the presence of Mn²⁺ in place of Mg²⁺. When 5'-(³²P)15-mer bound to mRNA was extended under these conditions, a series of products was obtained which were separated by polyacrylamide gel electrophoresis in the presence of 7 M urea. The length of these products as determined by comparison with single stranded DNA markers ranged from 15 to approximately 120 nucleotides, implying the presence of at least 80 nucleotides proximal to the initiation codon of this mRNA. Sequence analysis of the major bands by the new chemical technique of Gilbert and Maxam²⁰ has shown that the sequence of the short products is part of the sequence of the long products. The 14 nucleotide sequence which we have determined (Fig. 4), 5'-d(A-G-A-A-C-C-G-G-C-C-T-T-G-A)3', confirms the sequence Sherman and Stewart⁶ determined by genetic analysis (Fig. 1 *b* and *c*). This provides additional evidence that the synthetic 15-mer is binding specifically to the expected site on the yeast iso-I cytochrome *c* mRNA.

We are extending our studies to determine the sequence in the regulatory region of this yeast mRNA. The use of synthetic oligonucleotides as specific probes will make it possible to study many mRNAs which could not otherwise be approached.

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Anomaly in the γ chain heterogeneity of the newborn

THE two types of γ chain in human foetal haemoglobin (HbF) are the products of nonallelic structural genes and have either glycine (¹γ type) or alanine (²γ type) in position 136 (ref. 1). Although the examination of 108 cord blood samples from worldwide sources showed 102 to have a ¹γ : ²γ ratio within a relatively narrow range, one had a low ratio and five a high ratio². It seemed possible that

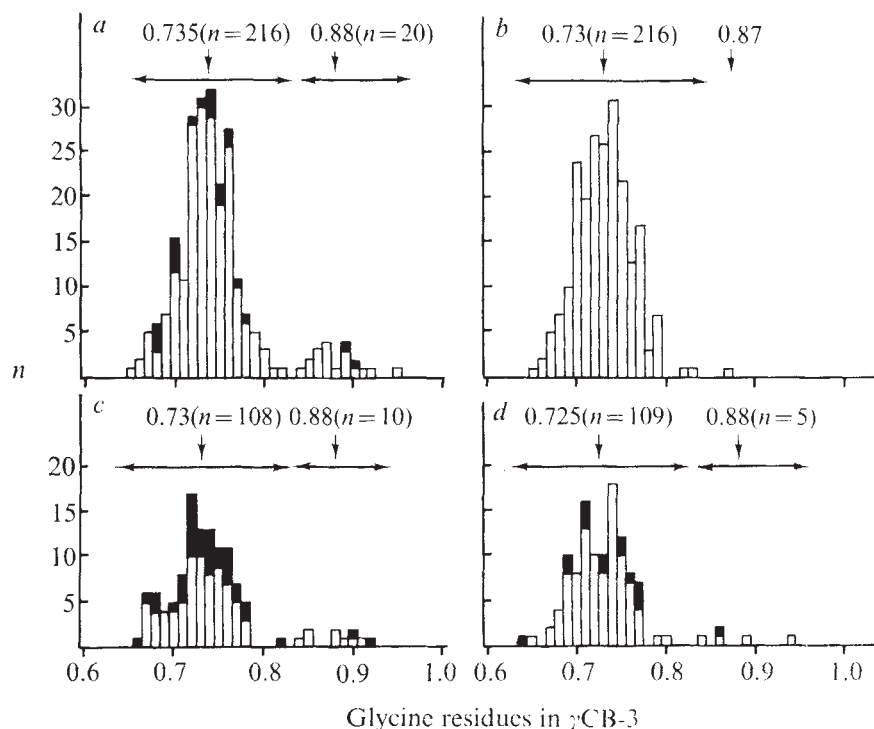


Fig. 1 Distribution of glycine residues of γ CB-3 from the cord bloods of black and Caucasian newborns from different areas. Open columns denote data from newborns without haemoglobinopathy, and black columns denote data from HbS- and/or HbC-containing samples. *a*, Burke County blacks; *b*, Caucasians; *c*, Ghana blacks; *d*, Los Angeles blacks.

analytical error was responsible for these extreme values. But as we studied cord blood from normal black babies of residents from Burke County, Georgia, where an abnormal HbF (designated F_x (ref. 3)) has been detected repeatedly, we observed high ratios in further samples. The present study of almost 700 cord bloods has detected the high $^{\circ}\gamma$: $^{\circ}\gamma$ ratio in about 7.5% of samples from black newborns as compared with perhaps 0.5% from Caucasian infants.

The nonallelic γ genes are found worldwide and are not limited to specific ethnic groups or geographical areas². Experimentally, the heterogeneity is best determined by cleaving the HbF with cyanogen bromide, isolating peptide γ CB-3, and making an amino acid analysis for glycine and alanine according to the procedure described previously¹. If the residues of glycine are integral at 1 or 0 and alanine at 2 or 3, the $^{\circ}\gamma$ or $^{\circ}\gamma$ chains alone are present in the HbF; a nonintegral number denotes a mixture. It is simplest to discuss results in terms of 'glycine values' rather than the $^{\circ}\gamma$: $^{\circ}\gamma$ ratio. Thus, in the initial study (2), 102 samples had glycine values between 0.65 and 0.79 residue; one was very low at 0.52 and five were above 0.8 residue.

The initial sampling of our extended study included only black children from Burke County. Subsequently, the survey was expanded to include blacks and whites from the Los Angeles area, blacks from Ghana, and whites from the Augusta area and from Malta.

That analytical error was not responsible for higher values was evident from reproducible data of 11 duplicate analyses which agreed with one exception within 0.05 residues as expected⁴.

The data are summarised in Fig. 1. Clearly the anomaly manifests itself almost exclusively in black infants of whom 35 (of 468 studied) have glycine values above 0.82 residue in contrast to Caucasian infants of whom perhaps only one (of 217 studied) has the effect. The seemingly different incidence of 8.5% among Burke County as well as Ghana black babies compared with 4.4% in Los Angeles area black newborns may be attributed to more settled and less migratory communities in Burke County and Ghana compared with the more cosmopolitan sources of blacks in the Los Angeles area. The incidence of the anomaly in infants with HbS and/or HbC is 7.1% (five out of 71 infants),

accords with the general incidence and suggests no relationship to an abnormal β gene.

Newborns with the anomaly are healthy babies without haematological problems. The range and average of alkali resistant haemoglobin by the Betke method⁵ was not significantly different in newborns who had glycine values above or below 0.82 residue. All nine mothers and fathers of newborns with the anomaly who were studied had normal haematological indices and percentages of HbA₂ in the normal range of 1.7–3.7% (ref. 6). Two mothers and one father had sickle cell trait, one father had AC trait, and one mother had a slightly elevated level of Hb-F (Hb-F_{AD}: 4.1%).

It has been postulated that there may be as many as four structural γ chain loci (two $^{\circ}\gamma$ and two $^{\circ}\gamma$ loci (ref. 3)). Recent evidence suggests only two or three loci⁷. Several regulatory factors seem to be interspersed among the complex of β , γ and δ genes to control the production of protein by each gene⁸. Neither the nature nor the mode of action of these regulatory factors is known. Perhaps, the anomaly exerts its action either by increasing the production of the gene(s) for the $^{\circ}\gamma$ chains or vice versa by decreasing the activity of gene(s) for the $^{\circ}\gamma$ chain. Incomplete investigations suggest that the anomaly is hereditary.

As the HbF decreases postnatally until only traces of HbF remain in the adult, the glycine value also changes from approximately 0.73 in the infant to 0.4 (range about 0.25–0.55) in the adult⁹. Does a high glycine value at birth remain high or does it change to the adult range? Conversely, can a 'normal' glycine value of about 0.7 at birth remain unchanged? It has indeed been observed in a newborn with sickle cell anaemia that the 'normal' glycine value did not fall to the adult range¹⁰. Perhaps the anomaly reported here is responsible for those adult cases of sickle cell anaemia in which, unexpectedly, the glycine value falls in the newborn range rather than in the adult range^{11,12}. Whether or not the recently reported $^{\circ}\gamma$ chain¹³ is involved remains to be determined. These problems are under investigation.

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Evidence for localisation of genes for human α -globin on the long arm of chromosome 4

THERE is disagreement about the site of the human haemoglobin structural genes, mapping of which has been attempted by *in situ* hybridisation¹⁻⁴ and family studies^{5,6}. After *in situ* hybridisation of human metaphase chromosomes with radioactive globin mRNA Price *et al.*¹ suggested that the haemoglobin structural genes are located on the long arm of chromosome 2 and on the long arm of a B-group chromosome, the latter carrying the genes for β , γ and δ chains. Their approach was criticised because of the low specific activity of the ³H-mRNA assay used^{7,8}, but Atwood *et al.*² and Price and Hirschhorn^{3,4} confirmed the original assignments using human complementary DNA (cDNA). Finally it was decided that the B-group chromosome that carries haemoglobin loci is chromosome 4^{3,4}. Family studies gave results consistent with loose linkage between the locus of the MN blood group and β -thalassaemia. The MN blood group has been mapped tentatively on the long arm of chromosome 2 or on the distal end of the long arm of chromosome 4 (refs 9, 10). But the localisation of the MN system on 2q is inconsistent with data from family studies¹¹⁻¹³, and the distal segment of 4q has been considered more probable¹¹. For similar reasons the β -globin locus has been mapped tentatively on chromosome 4 (ref. 5). We report here a study of globin synthesis which confirms that chromosome 4 is the site of the structural gene for α -globin.

We used reticulocytes from patients with congenital aberrations of chromosome 4, which had been demonstrated by

R, T, and BUdR-acridine orange banding^{14,15}. Case 1 (ref. 16) had duplication 4q28→4qter on the long arm of chromosome 2, associated with monosomy 2qter. The chromosomal formula was 46, XY, der (2), dup 4q, t(2;4) (q37; q28). In the healthy mother the same chromosome unbalance was detected in 10% of cells. Case 2 (ref. 16) showed duplication 4q28→4qter resulting from maternal translocation t(4; 18) (q28; q22), and was associated with monosomy 18qter. The chromosome designation was 48, XY, der(18), dup 4q, t(4;18) (q28;q22). Case 3 (ref. 16) displayed a "mirror" duplication of chromosome 4 from band q25 to band q34. The designation was 46, XX, der (4), 4qter → 4q 34 : 4q 34 → 4q25 : 4q 34 → qter. Case 4 (ref. 17) was carrier of duplication 4p13→4p16, associated with monosomy for the iuxta telomeric region of the long arm of chromosome 4. The designation was 46, XX, rec (4) dup p, inv(4)(p13;q35). The aberrant chromosome was interpreted as an aneusomic recombinant from the father who was heterozygous for a pericentric inversion of chromosome 4.

Red blood cells were drawn, washed and incubated for 1 h with S³H-leucine, as described by Clegg *et al.*¹⁹ and modified by Riede²⁰. Radioactivity and absorbance determinations and specific activity was calculated as described before¹⁸.

As Table 1 shows, results differing significantly from control values were obtained with cases 1, 2 and 3. Two globin synthesis determinations (cases 1 and 3) were also carried out by Dr A. Fantoni (CNEN, Rome), who obtained ratios of synthesis α and β of 1.4 for case 1 and 0.79 for case 3.

Since red cell morphology and haemoglobin A₂ levels of the four patients, and their parents were normal, we concluded that cases 1, 2 and 3 had unbalanced globin synthesis of non-thalassaemic origin (pseudo- β -thalassaemia in case 1 and 2, and pseudo- α -thalassaemia in case 3).

Human α and β globins are normally synthesised in equal quantities. But duplication of a chromosome fragment including one of the two globin genes could result in increased production of the globin encoded by the duplicated fragment, with consequent unbalanced globin synthesis.

In cases 1 and 2 part of the long arm of chromosome 4 (bands 28 to 35 included) was duplicated; in both cases an excess of α -globin synthesis was observed. In case 1 the ratio of α/β synthesis was 1.45, which was very close to expectation in erythroid cells with three doses of functional α genes instead of two. Therefore it seemed reasonable to postulate for the diploid cells of this patient the presence of two genes for β globin and, due to chromosome duplication, one additional dose of genes for α globin. Case 2, although morphologically identical to case 1, showed an excess of α -globin synthesis smaller than expected for functional trisomy of the α -globin genetic region. It is possible that in this case the translocated fragment was somehow less active in globin synthesis than normal. Anyway, both cases suggest the localisation of the genes for α globin on the long arm of chromosome 4 (from band 28 to 35). Band q35 is excluded by the data obtained for case 4 in which this region was deleted and the ratio of α/β globin synthesis was 1.0.

The chromosome abnormality of case 3 was the duplication and inversion of the long arm of chromosome 4 from band 25 to 34, including the same region duplicated in cases 1 and 2. In case 3, however, the ratio of synthesis of α and β globins was significantly below unity.

Observations of case 3 (personal communication from B. Dutrillaux) have shown that in 50-60% of lymphocytes cultivated in the presence of BUdR the long arm of the rearranged chromosome 4 is uncoiled and less fluorescent after acridine-orange staining. This suggests the genetic inactivation of the long arm of the chromosome. The suggestion is derived from studies correlating BUdR uncoiling and genetic inactivation in translocations involving the X chromosome²²⁻²⁷. In our case despiralisation could involve the segment carrying the α genes, resulting in their inactivation. The erythroid cells of the subject could be therefore a mosaic of two populations, one with three and the other (that with despiralisation) with one

Table 1 Synthesis of α and β globin in reticulocytes from the patients

Case	Chromosome unbalance	Radioactivity (c.p.m.)		Absorbance at 280 nm		Specific activity (c.p.m.)		Ratio of specific activities
		α	β	α	β	α	β	
1*	dup 4q28→4q35	19,450	18,866	18.6	25.9	1,045.6	728.4	1.43
2	dup 4q28→4q35	8,100	7,200	14.0	15.6	578.5	461.5	1.45†
3*	dup inv (4)q25→4q34	9,900	14,508	17.1	22.5	577.5	643.6	1.25
4	dup 4p13→4p 16, del 4q35	8,820	9,360	14.55	15.39	606.1	608.1	0.89
Control cases								0.79†
								1.00
								1.0±0.1

*Data obtained after rechromatography of the α - and β -globin regions.

†Data obtained in a different laboratory, by a different technique.

dose of functional α -globin gene. This would explain the slight defect of α -globin synthesis which we found. The cell population with α -gene inactivation should have a pronounced defect of α -globin synthesis (relative to β -globin synthesis) with consequent accumulation of β_4 tetramers (HbH).

A proportion of the red cells of this subject should therefore contain inclusion bodies. It has not yet been possible to demonstrate such inclusion bodies.

Duplication of the α -globin structural genes has been shown in man²⁷. It seems reasonable that the type of duplication fixed by evolution has allowed a balance between α and not α -globin chains; on the contrary this balance has not been respected in the chromosomal aberrations we have studied. On the other hand there is no evidence of chromosomal rearrangements deactivating the regulatory mechanisms of synthesis of structural genes, as observed in patients affected by Down's syndrome (Conconi and Del Senno, unpublished observations).

On the contrary there is considerable evidence for a dosage effect in the case of aneuploidy.

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Vitamin A receptors in normal and dystrophic human retina

VITAMIN A is necessary for normal development and functioning of most tissues of the body^{1,2}. It is uniquely important for the retina in that, in addition to its possible general role in cellular metabolism, it participates in the visual process as the prosthetic unit for rhodopsin. This takes place in specialised regions of the photoreceptor cell called rod outer segments (ROS). Little is known, however, about uptake, binding and translocation of retinol, the alcohol form of vitamin A, in the photoreceptor unit. A specific 2S receptor for retinol of molecular weight about 17,000 has been described in several animal tissues^{3,4}. Retinol receptors are also present in retina and pigment epithelium⁵⁻⁸. In addition, we have recently found that an 8S receptor is present in retina and brain of most but not all animal species tested⁹. We report here that normal human retina exhibits both a 2S and 8S binding species for retinol but that only the 2S species is observed in the retina of a patient who had retinitis pigmentosa (RP).

Eyes from two patients with normal retinæ and from a patient with retinitis pigmentosa were maintained at 4 °C and used within 4 h of death. The two patients with normal retinæ had died of heart disease (48 yr old) and acute lymphocytic leukaemia (17-yr old). The patient with retinitis pigmentosa died from bronchogenic carcinoma at 59 yr. He had extensive loss of peripheral vision, a characteristic finding in the disease. The retinitis pigmentosa in his family is consistent with either the X-linked intermediate or the autosomal dominant type. For histological and electron microscopic examination, a portion of the retinal tissue was fixed in 4% glutaraldehyde in 0.15 M phosphate buffer (pH 7.2) and sections were examined with a JEM-100B electron microscope. For biochemical analysis, retina and

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pigment epithelium-choroid layers were dissected individually, homogenised in 10 mM Tris buffer (pH 7.6, containing 1 mM EDTA, 10 mM KCl and 1 mM dithiothreitol) and the 110,000g supernatant fraction (cytosol) was prepared⁵. Portions (0.25 ml) of the cytosol were incubated with 1×10^{-7} M ^3H -retinol (2.66 Ci mmol⁻¹, New England Nuclear) for 2 h at 4 °C. All operations involving vitamin A were performed in darkness or under dim red light. Samples were layered on 4.6 ml linear sucrose gradients (5–20% sucrose in the Tris buffer) and centrifuged 16 h at 243,000g. Approximately 35 fractions were collected per gradient and radioactivity determined as previously described⁵. Protein was measured by the method of Lowry *et al.*¹⁰. Concentrations were 6.1 and 5.0 mg protein ml⁻¹ for normal and RP retinal cytosol samples and 5.6 and 2.0 mg protein ml⁻¹ for normal and RP pigment epithelium-choroid cytosol.

Morphologically, the RP retina and pigment epithelium exhibited degenerative changes with marked loss of photoreceptor cells. Appreciable numbers of cone cells remained in the macula, but few of these had outer segments (Fig. 1a). Outer segments were detected only in the foveal area and were considerably disorganised as assessed by electron microscopy (Fig. 1b). Thus the sample was typical of advanced retinitis pigmentosa.

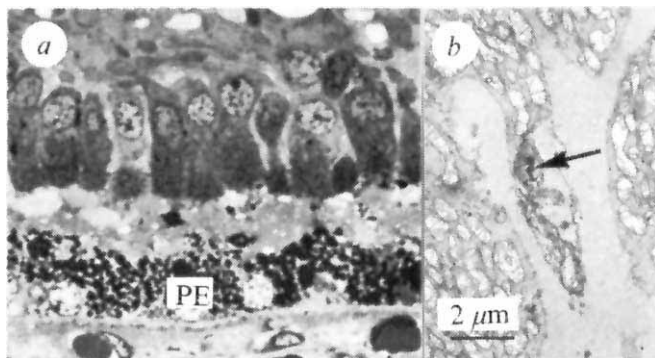


Fig. 1 *a*, Photoreceptor cells and pigment epithelial cells in the parafoveal area ($\times 660$). Although the outer segments are generally missing in the area, cone cell bodies (C) are still present. The pigment epithelial cells (PE) contain abundant lipofuscin granules. *b*, Disorganised lamellar membranes within the outer segment of a cone cell are still recognisable (arrow) in the foveal area ($\times 4,320$).

As assessed by sucrose density gradient centrifugation, two binding species for ^3H -retinol were observed in the cytosol fraction of normal human retina (Fig. 2a). Sedimentation coefficients are approximately 2S (peak at fraction 26) and 8S (peak at fraction 13). Under the present conditions, 180 and 900 fmol of vitamin A were bound per mg of total protein in the 2S and 8S peaks respectively. In the cytosol fraction of the RP retina, the 8S peak was completely absent. The 2S peak was comparable with that seen in the normal retina with 240 fmol of vitamin A bound per mg protein.

With normal pigment epithelium-choroid cytosol, a small 8S peak was seen along with a larger 2S peak (Fig. 2b). A 2S peak was observed in the cytosol fraction from the RP eye but the 8S peak seemed to be absent. Since the protein concentration was lower in the RP cytosol sample, binding in the 2S region of RP pigment epithelium-choroid cytosol was actually somewhat higher than in the normal case with 590 fmol and 800 fmol vitamin A bound per mg protein for normal and RP cytosol samples respectively. The 8S peak observed in the pigment epithelium-choroid preparation may be endogenous to the pigment epithelium or alternatively could be ascribed to ROS contamination. Even using the most favourable conditions, when normal retina is separated from pigment epithelium, a variable percentage

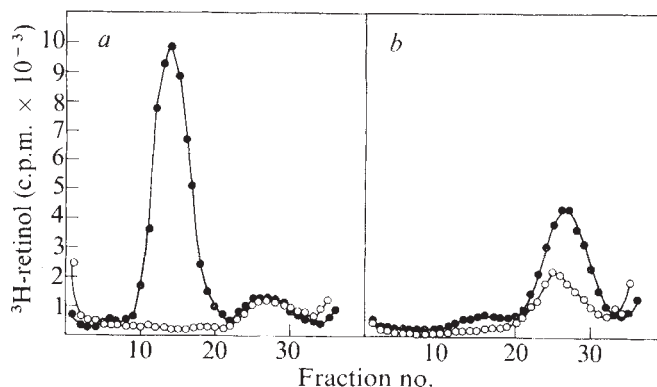


Fig. 2 *a*, Sucrose density gradient centrifugation patterns of human retinal cytosol fraction incubated with 0.1 μM ^3H -retinol. $\bullet$, Normal retina; $\circ$, RP retina. *b*, Patterns with human pigment epithelium-choroid cytosol fraction incubated with 0.1 μM ^3H -retinol. $\bullet$, Normal pigment epithelium-choroid; $\circ$, RP pigment epithelium-choroid.

of photoreceptor tips breaks off and remains entrapped in the microvilli of the pigment epithelium. In the RP eye, the lack of this small 8S peak may then be correlated with the virtual absence of ROS in the retina and gives supportive evidence for compartmentalisation or concentration of the 8S receptor species in ROS. Attempts to identify retinol receptors in purified ROS preparations have so far been unsuccessful, most probably because the receptors are soluble and are easily lost from ROS membranes during purification procedures.

These data indicate that not only does the human retina contain two distinct receptor species for retinol, but that these may be compartmentalised in different retinal areas. If one assumes that the 8S species is solely associated with outer segments, one would then expect an absence of this receptor species in the severely affected RP retina examined. In this way the RP retina serves as a model for a 'rodless' retina. Sequestering of the 8S species in an organelle as specialised as the photoreceptor unit would strongly implicate the receptor in the visual cycle, possibly as a transport vehicle for vitamin A between retina and pigment epithelium.

Lack of the 8S binding species may also be correlated with a specific biochemical abnormality resulting in or genetically linked to retinitis pigmentosa. Study of the receptors both qualitatively and quantitatively in retinæ from young patients if they become available for research will clarify this point. In any event, the presence of at least two receptor species for retinol in normal human retina and the possible compartmentalisation of the 8S species in the photoreceptor unit suggest an important role for such receptors in normal retina functioning.

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Identification of a membrane protein binding the retinol in retinal pigment epithelium

AVAILABLE experimental evidence indicates the primary role of retinal pigment epithelium (PE) in the uptake of retinol from the extravascular space of the choriocapillaris¹ and possibly in its transfer to and from the photoreceptors². While the transport mechanism of retinol in plasma by the retinol-binding protein (RBP) is known in detail^{3,4}, only partial information is available on how circulating retinol is transferred from its carrier protein to PE. It has been shown⁵ recently that iodinated human RBP binds specifically to isolated bovine PE and autoradiographic investigations⁶ have suggested that the binding is confined to the choroidal surface of these cells. We have previously demonstrated⁷ that isolated human and bovine PE actively binds ³H-retinol only if this is present in the incubation medium as a complex with human RBP, and suggested that the interaction of circulating RBP with the cell membranes of PE is specific (G.M. and F.G., unpublished observations), and essential, for the active transport of retinol in these cells against a concentration gradient. We have now identified, in the plasma membranes of bovine PE incubated in the presence of ³H-retinol-RBP, a binding protein for retinol; some evidence is presented which suggests that this membrane protein is different from plasma RBP.

The RBP used in these experiments was prepared from human plasma as described by Peterson and Berggard⁴ and purified by affinity chromatography on prealbumin-coupled Sepharose 4B according to Vahlquist *et al.*⁸ Retinol was then extracted from the lyophilised RBP with ethanol and ³H-retinol (specific activity 2.4 Ci mmol⁻¹) substituted for the original chromophore in the apo-RBP following the procedure described in detail by Heller and Horwitz⁹. The reconstituted ³H-retinol-RBP was again purified on the same affinity chromatography column in order to eliminate excess ³H-retinol and possible residual apo-RBP by utilising the inability of retinol-free RBP to bind prealbumin^{8,10}.

PE was prepared from bovine eyes immediately after death as previously described⁷. Such PE preparations show no appreciable contamination by rod outer segments nor by choroidal remnants, and they actively synthesise proteins in short term (2 h) incubation experiments⁷. After the last washing the PE pellet was carefully resuspended in 2 ml of the same medium containing ³H-retinol-RBP (0.1 nmol ml⁻¹) and incubated for 1 h at 37 °C in a water bath with occasional gentle stirring. At the end of the incubation period the cells were separated by centrifuging, washed twice in ice-cold unlabelled medium, homogenised in 1 ml of medium with a tight-fitting Teflon glass homogeniser and separated on a discontinuous sucrose gradient. By this method it was possible, as shown by electron microscopy of fixed samples of the different bands collected at the gradient interfaces, to separate the plasma membranes of PE clearly from the soluble cell sap and from the other cellular organelles. The gradient was then scanned at 280 nm and tested for radioactivity. The gradient pattern and the distribution of radioactivity of a typical experiment are reproduced in Fig. 1. Inspection of the figure shows clearly that radioactivity is practically limited to the two most superficial gradient bands, that is, those corresponding to the soluble cell sap and to the plasma membranes of PE. Although the ratio between turbidity and radioactivity is substantially similar for the two peaks, as much as 75% of the radioactivity is found in the plasma membrane fraction.

The fractions corresponding to this main peak were pooled and diluted with Krebs Ringer solution and the plasma membranes were collected by centrifuging at 35,000 r.p.m. in a SW40 rotor for 2 h. This washing procedure

did not cause the removal of significant amounts of the membrane-bound retinol since only about 6% of the original radioactivity was found in the resulting supernatant fluid. The plasma membrane pellet was then dissolved in a detergent solution and analysed for protein and radioactivity distribution by polyacrylamide SDS electrophoresis (7.5–12% acrylamide). The gels were calibrated for molecular weight determination using serum albumin (molecular weight 40,000), soybean trypsin inhibitor (molecular weight 20,000), human RBP (molecular weight 21,000), myoglobin (molecular weight 17,000) and cytochrome c (molecular weight 11,700).

Of the different detergent solutions used to solubilise the membrane preparations, 2.5% sodium dodecyl sulphate (SDS) was found to give the best recovery in the gels (over 90% of the radioactivity originally present in the sample). In more damaging conditions (4% SDS) this recovery was only 5%.

The gel staining pattern and the distribution of radioactivity indicate that all the ³H-retinol present in the gel is bound to a single Coomassie-blue stained band. Typical results are reproduced in Fig. 2. The electrophoretic mobility of this protein band is higher than that of native human RBP and indicates a molecular weight of approximately 14,500. This band follows in the gel a minor Coomassie-blue staining band which has a molecular weight

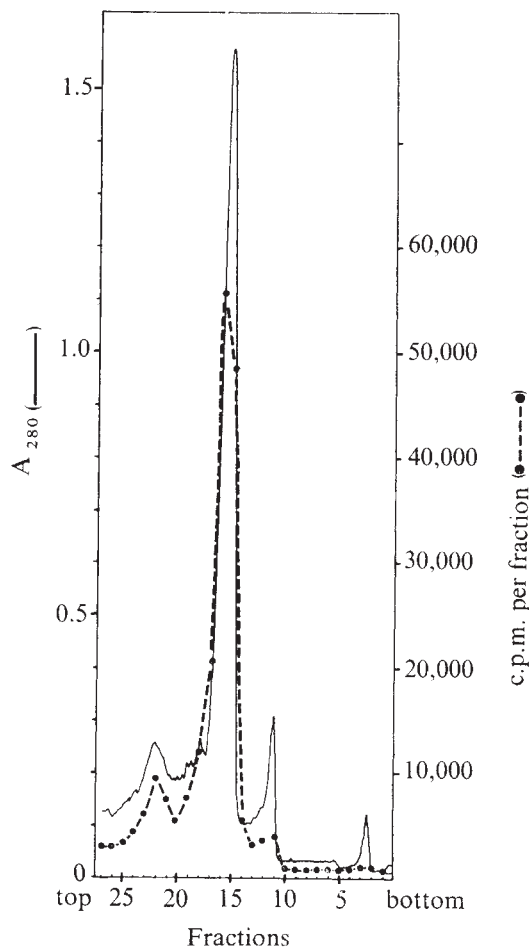


Fig. 1 Scanning and labelling profiles of homogenate of bovine PE preincubated with ³H-retino-RBP and fractionated on a discontinuous sucrose gradient by centrifuging for 150 min at 27,000 r.p.m. in a SW 40 rotor with a L₂ Spinco ultracentrifuge. The following sucrose solutions were layered in the centrifuge tube from bottom to top: 44%, 40%, 36%, 33%, 15%. The gradient was scanned at 280 nm utilising a LKB pump and a Pye-Unicam SP 1700 spectrophotometer. Fractions of 0.5 ml were collected. 15 µl aliquots of each fraction were tested for radioactivity in a Tricarb scintillation spectrometer (model 2425).

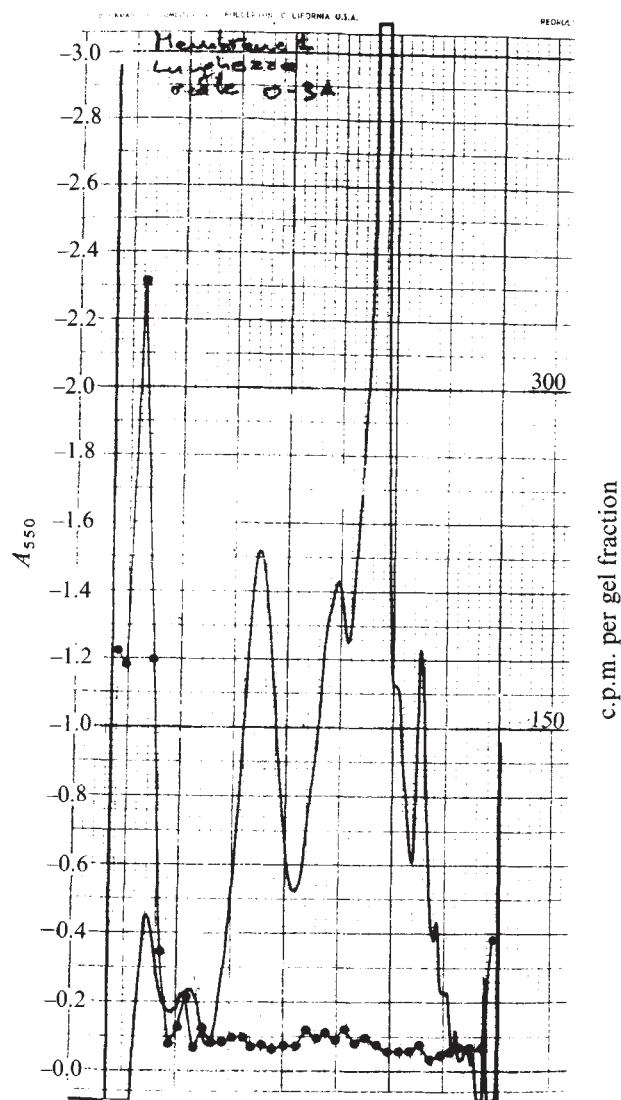


Fig. 2 Staining and labelling profiles of plasma membrane proteins from bovine PE preincubated with ^3H -retinol-RBP. Plasma membranes were dissolved overnight at room temperature with 2.5% SDS and 1% dithiothreitol in 0.3 M Tris-Cl⁻ buffer (pH 8.9) and analysed for protein and radioactivity distribution by polyacrylamide SDS gel electrophoresis. The gels contained 7.5% acrylamide (stock solution 30% acrylamide, 0.8% *n*-*n'*-methylene-bis-acrylamide). The amount of protein applied to each gel was 50 μg . Gels were fixed and stained with Coomassie-blue. Scanning of the gels was performed at 550 nm. Duplicate unstained gel was sliced into 2.3 mm fractions, dissolved in H_2O_2 at 45 $^\circ\text{C}$ for 2 d, and resuspended in Toluene 70%. Omnifluor 0.4% methylcellulose 30%. Radioactivity was determined by using a Tricarb scintillation spectrometer, with a counting error less than 1%.

of approximately 20,000 but which does not appear to contain appreciable radioactivity.

From the stained gel, after scanning, the protein band containing radioactivity was cut off. The gel slice was extracted three times with methanol and chloroform. The pool of the extracts was concentrated, applied on a thin layer of silica-gel F 254 and developed in the dark with a mixture of cyclohexane-ethanol 97/3 (ref. 11). Under ultra-violet light the chromatogram for reference retinol presented a principal spot with $R_f=0.51$ and two minor spots with $R_f=0$ and $R_f=0.95$ (oxidation products, probably the acid and the aldehyde). The sample zones corresponding to the three reference spots were scraped off, put in vials using 4% (w/v) Cab-O-Sil as gelling agent, and counted. Pure silica gel from the plate was scraped off as a blank. Radioactivity was present only in the principal spot ($R_f=0.51$); recovery of the gel radioactivity was 18%.

In four experiments isolated PE was incubated in the presence of iodinated human plasma RBP. Iodination of native RBP with ^{125}I sodium iodine and chloramine-T was performed as described by Heller³. After incubation with ^{125}I -RBP PE cells were treated as previously described. In none of the experiments could appreciable radioactivity be detected in the plasma membrane pellet at the end of the isolation procedure.

The gel staining pattern of plasma membranes of native PE (that is, not preincubated in the presence of RBP) is identical to that reproduced in Fig. 2 indicating that, under the experimental conditions used, no detectable change in the membrane protein composition was caused by the incubation with RBP.

Present results indicate that a protein able to bind retinol maybe extracted from the plasma membranes of PE incubated in the presence of exogenous RBP and is probably involved in the process of active retinol uptake by these cells. The molecular weight of this protein, calculated by SDS gel electrophoresis according to Weber and Osborn¹², is of the order of 14,500. This observation together with our inability to detect appreciable amounts of ^{125}I -RBP in the membrane preparation at the end of the isolation procedure suggests that the retinol-binding protein extracted from PE membranes is not the exogenous RBP present in the incubation solution. The same protein band was identified in SDS extracts of native PE membranes, which suggests that this protein is a normal constituent of this membrane. Nothing can be said at present about the possible relationship of this membrane protein with the low molecular weight retinol-binding proteins (molecular weight 17,000) recently demonstrated¹³ in the cytosol fraction of PE.

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Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells

OESTROGENS are more readily accumulated and retained in responsive cells than in cells that are not their targets¹. Cytoplasmic macromolecules² which specifically interact with oestradiol and other steroid hormones seem to mediate transfer of the agonist to the nuclear chromatin, where the complex is believed to promote expression of the phenotypic effects³⁻⁶. It is generally assumed that the hormone diffuses passively to "cytoplasmic" receptors which determine the cellular specificity of response⁷. But some experiments indicate that steroid hormones interact with components of biological membranes and may enter

their respective target cells by a membrane-mediated process⁸⁻¹² which is saturable and temperature-dependent¹³⁻¹⁷. We have investigated steroid-binding components associated with the plasma membranes of cells isolated from endometrium, liver and intestinal mucosa. Endometrial and liver cells show substantial binding to oestrogen immobilised by covalent linkage to an inert support, while intestinal cells have no such binding sites. The relative quantity of these steroid receptors at the outer surfaces of cells from diverse tissues corresponds well with the capacity of a given cell to accumulate and retain oestrogen.

Uterus, liver and intestine were excised from female rats that had been ovariectomised at 6 weeks old and maintained for 3 weeks in a low-steroid environment¹⁰. Epithelial cells were isolated from the mucosal surfaces of uterus and intestine by methods described before¹⁸. In brief, the procedures involved incubation with 20 mg of collagenase (Type I, Worthington) per 100 ml of Ca^{2+} , Mg^{2+} -free Ringer, followed by restoration of divalent cations. Complete Ringer solution consisted of 136.9 mM NaCl, 2.7 mM KCl, 0.6 mM CaCl_2 , 0.5 mM MgCl_2 , buffered at pH 7.4 with 8.1 mM Na_2HPO_4 : 1.5 mM KH_2PO_4 . Liver cells were isolated from excised tissues essentially by the method of Howard and Pesch¹⁹ with 200 mg of collagenase per 100 ml of the above media. Cellular debris and damaged cells were removed from the cell suspensions as described before^{12,18}. As a restorative procedure, the cells were incubated for 120 min in complete Ringer solution enriched with 0.1% (w/v) highly purified albumin (Pentex) and 1 mM sodium pyruvate before the start of experiments. Throughout these and all subsequent manipulations, at least 90% of all cells excluded the vital dye, Trypan blue, at a final concentration of 0.05% in Ringer solution during a 10-min incubation at 22 °C.

For determinations of total cellular uptake of free oestradiol, cells were diluted with fresh Ringer solution to a final concentration of 1×10^7 cells per ml. Incubations (5–60 min) were begun with the addition of 6, 7-³H-oestradiol-17 β (99 Ci mmol⁻¹, New England Nuclear; final concentration, 1×10^{-9} M) at 22 °C in oxygen. A 200-fold excess of unlabelled oestradiol-17 β ($\text{E}_2\beta$) was added to paired samples for determination of displaceable or non-specific binding¹⁴. Incubations were terminated by dilution with 4 volumes of ice-cold Ringer solution. The cells were then sedimented by centrifugation at 800 *g* (4 °C) for 5 min. The resulting cell pellets were washed three times by resuspending them in ice-cold Ringer solution and centrifuging as before. The washed pellets were sonicated at 0–4 °C, and the radioactivity in samples of the disrupted cells was determined by liquid scintillation spectrophotometry with 40–43% efficiency. Additional aliquots of the sonicate were analysed by the method of Hill and Whately²⁰ for DNA.

To prepare oestrogen-derivatised supports for determinations of cell binding, fibres of transparent nylon monofilament (size 50, Penn Products), were strung into polyethylene rings (cut from S-6 stoppers, Mallinckrodt) as described by Edelman *et al.*²¹. The mounted fibres (8 mg nylon per ring), in individual Petri dishes (10 × 35 mm), were extracted to remove surface contaminants for successive 10-min periods with petroleum ether, carbon tetrachloride, 80% (v/v) methanol and water. The surface of the nylon was then partially hydrolysed with 3 N HCl for 30 min at 30 °C²². After thorough washing with water to arrest further hydrolysis, the fibres were activated with 12.5% (v/v) glutaraldehyde (grade I, Sigma) in 0.1 M phosphate buffer, pH 7.4, at 37 °C for 24 h (ref. 23). The reaction was terminated by exhaustive washing of the fibres with the buffer. The activated fibres were next in phosphate buffer and incubated for 18 h at 4 °C. Fibres placed in Petri dishes containing 5 ml of 2% (w/v) albumin

were then washed with buffer to remove albumin that was not covalently bound to the activated nylon²³. Samples of the combined washes were taken to determine protein by the method of Lowry *et al.*²⁴. The introduction of albumin on the fibre was 0.12 mg per mg of nylon.

Oestradiol hemisuccinate was coupled to the albumin: nylon fibre with carbodiimide by procedures similar to those described by Sica *et al.*²⁵. The protein-derivatised fibres were transferred to 20-ml beakers in which were placed 10 ml of 70% (v/v) aqueous dioxane containing 17 β -oestradiol 17-hemisuccinate (272 μg per mg of albumin, Sigma). Two 50-mg portions of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Aldrich) were added (at 0 and 4 h). The samples were rocked gently for 18 h at 22 °C. The fibres were then washed at the same temperature successively with a total volume of 500 ml of dioxane (1 h), 51 of 80% methanol (72 h), and 500 ml of water (15 min). The oestrogen-derivatised fibres were transferred to fresh Petri dishes, dried and stored *in vacuo* at 0–4 °C until use. The amount of covalently bound oestrogen was determined in three samples by incubation of derivatised fibres (0.36 mg albumin) with 2 ml of 0.5 M NaOH at 22 °C for 2 h. After stoichiometric neutralisation with HCl, the free oestradiol was

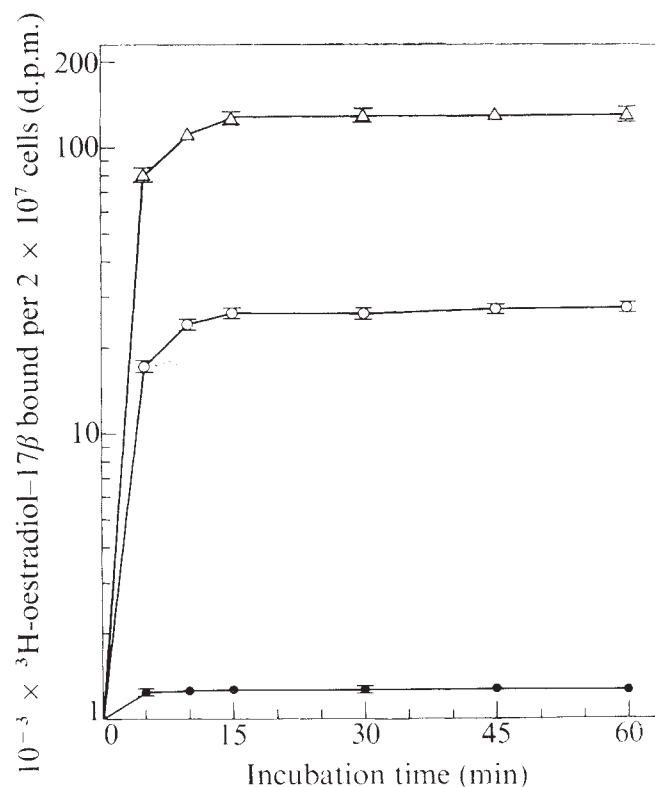


Fig. 1 Uptake of ³H-oestradiol-17 β by intact cells. Cells isolated from intestinal mucosa (●), liver (○) and endometrium (△) were incubated at 22 °C for 5–60 min with 1×10^{-9} M ³H- $\text{E}_2\beta$ as described in the text. Only specific binding (defined as the difference in bound steroid between paired tubes, one of which contained a 200-fold excess of unlabelled $\text{E}_2\beta$ throughout the experiment) is shown. Specific binding of $\text{E}_2\beta$ by liver and endometrial cells (22 °C, 30 min) was not reduced significantly in the presence of the relatively inert oestradiol-17 α at a concentration of 2×10^{-9} M ($P > 0.80$). Among liver and endometrial cells incubated at 4 °C for 30 min with 1×10^{-9} M ³H- $\text{E}_2\beta$, specific oestrogen binding was reduced to 26% of that bound to paired cells maintained at 22 °C (both at $P < 0.01$, Student's *t* test). The extent of $\text{E}_2\beta$ binding is expressed relative to cell numbers, as derived from determinations of cellular DNA content (for example, 8.3, 10.6 and 9.3 μg of DNA per 10^6 cells of endometrium, liver and intestinal epithelium, respectively). Detailed analyses of specific oestrogen uptake, to be presented elsewhere, indicate that binding sites per endometrial, liver and intestinal mucosal cell average $\sim 24,000$, 3,400 and 50, respectively.

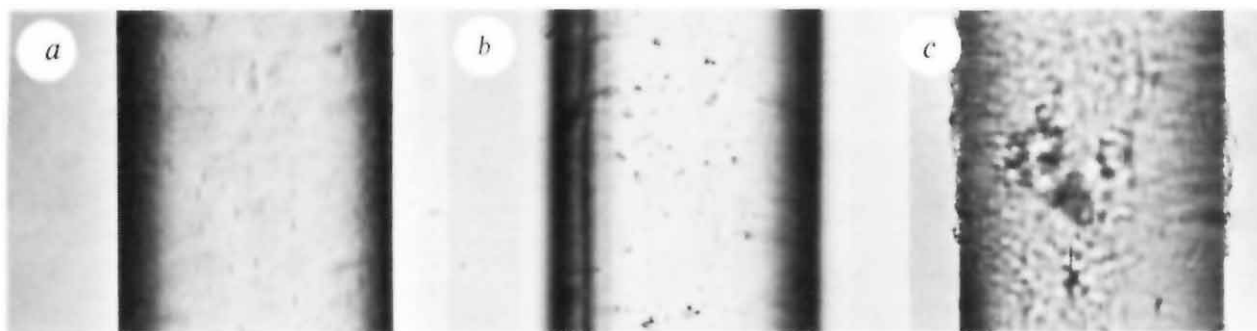


Fig. 2 Binding of isolated cells to oestrogen immobilised by covalent linkage to albumin-derivatised nylon fibres. Cells derived from intestinal mucosa (a), liver (b) and endometrium (c) were incubated with the derivatised fibres by addition of 1×10^7 cells per 4 ml of Ca^{2+} -, Mg^{2+} -free Ringer solution to a 10×35 -mm Petri dish with the mounted fibres (8 mg of nylon per dish). The dishes were placed on a platform shaker (80 oscillations per min) at 22°C for 30 min, after which unbound cells were separated by complete immersion of the dish successively in a series of three beakers, each containing 10 volumes of the incubation medium. The washed fibres with retained cells were then transferred to 4 ml of fresh incubation medium for photomicrography, using an immersion lens and Kodak Tri-X Panatomic film (ASA 400). Significant numbers of hormone-responsive cells (that is endometrial and liver cells) adhere to the fibres, whereas "nontarget" cells from intestine are poorly retained. No binding of any cells was observed when underderivatised fibres or fibres coupled only to albumin were used ($\times 250$).

extracted with four equal volumes of diethyl ether (Mallinckrodt) that had been freshly saturated with double distilled water. The combined ether extracts were repeatedly backwashed with water, and evaporated at room temperature with a stream of nitrogen. Oestrogen was determined in the resultant residue by radioimmunoassay²⁶ (courtesy of Dr R. Swerdloff). The minimal substitution of oestradiol was 1 ng per mg of albumin.

Figure 1 shows the extent of uptake of 1×10^{-8} M $^3\text{H-E}_2\beta$ by intact cells isolated from endometrium, liver and intestinal mucosa. The specific accumulation reported here represents the amount of radioactivity not displaced by the addition of a 200-fold excess of unlabelled $\text{E}_2\beta$. Among all cells incubated at 22°C , accumulation of $\text{E}_2\beta$ was essentially maximal within 10–15 min, and there was no further increment in uptake of steroid for up to 60 min of incubation. As anticipated, endometrial cells retained the most $\text{E}_2\beta$ per cell. The moderate level of hormone uptake observed in liver cells is consistent with evidence for the presence of specific oestrogen receptors in rat liver^{27,28}. The least retention was seen with epithelial cells from intestine, which is considered not to be a target tissue for oestrogen²⁹. In addition, $\text{E}_2\beta$ binding to liver and endometrial cells maintained at 4°C for 30 min was approximately four times less than that bound to corresponding cells incubated in the same conditions, but at 22°C (Fig. 1).

To probe for steroid-binding sites at the external surfaces of cells derived from endometrium, liver and intestinal mucosa, isolated cells were incubated with the mounted 17β -oestradiol-17-hemisuccinyl: albumin: nylon fibres in the standardised conditions indicated in Fig. 2. Cells retained by the oestrogen-derivatised fibres after extensive washing were observed by microscopy. Significant numbers of hormone-responsive endometrial and liver cells were found to adhere to the steroid-derivatised fibres, but nontarget cells from intestine were essentially not retained (Fig. 2). Since there was no association of cells when unmodified nylon fibres or fibres derivatised only with albumin were used, the binding to oestrogen-derivatised fibres by target cells exclusively must be attributable to the presence of the steroid and the occurrence of specific recognition sites at the external surfaces of given cells.

A quantitative estimate of cell binding to the derivatised fibres was obtained as described in Fig. 3. Most fibre-bound cells could be displaced and recovered free and apparently intact by competitive inhibition of binding with excess $\text{E}_2\beta$, but not with oestradiol- 17α ($\text{E}_2\alpha$) added to the medium. Remaining cells were dislodged by brief exposure to hypotonic saline²¹. Among all cell types, the number of bound

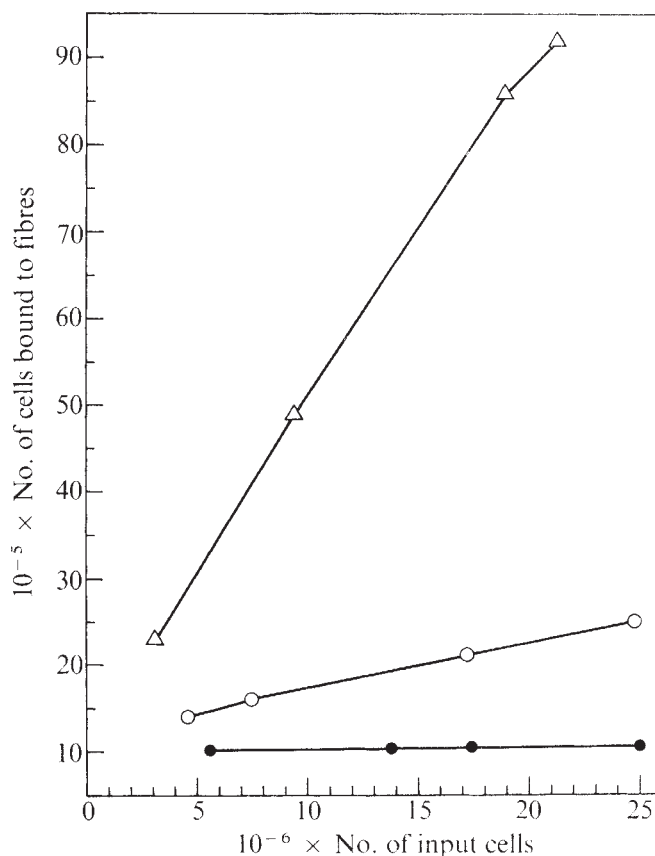


Fig. 3 Extent of cell binding to oestrogen immobilised on nylon fibres. Cells isolated from intestinal mucosa ($\bullet$), liver ($\circ$) and endometrium (Δ) were incubated with the fibres by the addition of 3.25×10^6 cells per 4 ml of medium using standard conditions of mixing and shaking (Fig. 2). After removal of unbound cells, cells attached to the derivatised fibres were dislodged by incubation for 1 h in divalent cation-free Ringer solution containing 2×10^{-7} M $\text{E}_2\beta$, followed by 1 min of incubation in hypotonic (150 mosm) saline²⁵ with excess $\text{E}_2\beta$. ($\text{E}_2\beta$ was ineffective in displacing cells from their association with fibres.) Cells recovered by these procedures were disrupted by sonication at 4°C , and samples of the sonicate were taken for determination of cellular DNA contents. Cell numbers reported here were derived from measurements of DNA content per cell (Fig. 1). Each data point represents the mean of two or three independent experiments. The relation of the number of fibre-bound cells to total cells present in the incubation medium is defined by the slope, 0.003 for intestinal cells ($r = 0.98$), 0.054 for liver cells ($r = 0.99$), and 0.386 for endometrial cells ($r = 0.99$).

cells was a linear function of the cell concentration over a wide range (Fig. 3). This result suggests that, in the conditions specified, binding is proportional to the number of input cells; the binding of one cell is independent of that of others, and the fibre surface is not saturated with cells. As in analyses of total cellular uptake of $E_2\beta$ (Fig. 1), the capacity of endometrial cells to bind oestrogen coupled to a fibre surface surpassed that of cells from liver and intestine. The percentage of total input cells that become bound averaged 40 ± 1 (9) for endometrial cells, 7 ± 1 (8) for liver cells, and 0.3 ± 0.1 (8) for intestinal epithelial cells.

The number of endometrial cells bound to derivatised fibres was significantly reduced by incubation at low temperature and also by exposure of cells to free $E_2\beta$ followed by three washes at 400g, before incubation of the cells with fibres in the standard conditions (Table 1). Binding of oestrogen at the outer surfaces of cells incubated at 4 °C was four times less than that to paired cells maintained at 22 °C. Association of $E_2\beta$ -pretreated cells with derivatised fibres (at 22 °C) amounted to 42% of that of corresponding endometrial cells exposed only to hormone vehicle, ethanol, at a final concentration of 0.02% ($P < 0.01$). In contrast, prior incubation of these cells with $E_2\alpha$ elicited no significant reduction in the subsequent binding of washed cells to the test fibres compared with that of control cells ($P > 0.90$).

These studies provide direct evidence that receptor components specific for oestradiol-17 β , but not for the 17 α -isomer, are present at the external surfaces of oestrogen-responsive cells. These results seem to be in conflict with the assumption that steroid hormones enter all cells by nonspecific, free diffusion^{6,30}. Several investigators, however, have noted marked saturability and temperature-dependence of steroid hormone entry, which cannot be attributed to the function of cytoplasmic receptors¹³⁻¹⁷. Our studies are consistent with these observations. Moreover, the decline in oestrogen accumulation by target cells at reduced temperature may be attributable to a reduction in the availability of surface receptors for the hormone. Alterations in the physical state and orientation of various membrane macromolecules may contribute to the reduced interaction of oestrogen with surface binding components at low temperatures^{12,31-33}.

The number of surface sites available for binding to oestrogen-derivatised fibres is markedly diminished in endometrial cells after exposure to oestradiol-17 β , but not after treatment with the relatively inactive 17 α -isomer. This may be attributable to masking of the external sites

by bound oestrogen or to a hormone-induced change in the location or orientation of the oestrogen-binding components. Other studies have shown that target-selective uptake of specific antigens is associated with localised dis-ordering of membrane structure and subsequent internalisation, presumably of those regions of the surface membrane equipped with, or immediately adjacent to, specific recognition sites³⁴⁻³⁶. A corresponding sequence of events may also occur at the surfaces of endometrial cells in response to oestrogen, as is evident from reports of altered membrane functions^{12,37,38} and micropinocytotic vacuolation of the plasmalemma³⁵ after brief exposure to oestrogen.

In contrast to numerous reports devoted to the identification of membrane-associated receptors for polypeptide hormones^{39,40}, membrane-binding sites with specificities for steroid hormones have scarcely been investigated. Our work provides direct proof that such sites exist at the surface membrane of cells responsive to oestrogen. But the nature and origin of these binding components and the manner in which they may contribute to the recognition and mediation of entry of oestrogenic hormones remain to be determined.

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Table 1 Effect of temperature and prior treatment with oestrogens on the binding of isolated endometrial cells to oestrogen immobilised by covalent linkage to albumin-derivatised nylon fibres

Incubation temperature (°C)	Treatment before incubation	No. of fibre-bound endometrial cells number of input cells (%)
22	None	40 ± 1 (3)
4	None	10 ± 1 (3)*
22	Oestradiol-17 α (1×10^{-9} M, 1 h)	39 ± 2 (3)
22	Oestradiol-17 β (1×10^{-9} M, 1 h)	17 ± 2 (3)†

The extent of endometrial cell binding to oestrogen-derivatised fibres at 22 °C or 4 °C was determined as described in Figs 2 and 3. The number of cells added to each incubation vessel, the input cells, averaged 2×10^7 ($\approx 166 \mu\text{g}$ DNA). In some experiments, cells were treated for 1 h with 1×10^{-9} M $E_2\beta$ or $E_2\alpha$ and then washed three times in steroid-free medium at 400g (5 min) before exposure to the derivatised fibres in the standardised conditions. All data are presented as mean $\pm$ s.e.m. with the number of determinations in parentheses.

* Value significantly different from appropriate control at $P < 0.001$.

† Value significantly different from appropriate control at $P < 0.01$ (Student's *t* test).

Abnormal myelination in transplanted Trembler mouse Schwann cells

AXON and Schwann cell interdependence has received increasing attention in recent years^{1,2}. In normal nerves, axons influence Schwann cell proliferation^{3,4} and myelin formation⁵⁻⁸. These interactions may also be important in peripheral nerve disorders. Indeed, it has been suggested that in certain neuropathies, it is an axonal abnormality which is responsible for the failure of Schwann cells to maintain normal myelin^{9,10}. An experimental approach to this question has become possible with the demonstration that, in nerve grafts from normal animals, Schwann cells which originate in donor nerves ensheath and myelinate axons arising from nerve cells in the host^{6,8,11}. This demonstration of the feasibility of *in vivo* combinations of Schwann cells and axons, each originating from different animals, prompted the present experiments in which axons and Schwann cells from normal and abnormal mice were combined in sciatic nerve grafts. For the present study, abnormal nerves were obtained from Trembler mice (Tr)¹². These mutants have a dominantly inherited neuropathy characterised by the presence of abnormally thin myelin sheaths and slow conduction in the peripheral nerves¹³, changes which have been considered to resemble those of human hypertrophic neuropathies¹⁴.

To define further the abnormality in Tr neuropathy, sciatic nerves and spinal roots from six affected mice and five control C57BL/6J mice aged 14 d to 7 month were examined by phase and electron microscopy^{6,15}. In normal nerves, most axons greater than 1 μ m in diameter were surrounded by Schwann cells containing a myelin sheath; in Tr nerves, between 30 and 70% of Schwann cells ensheathing fibres of this size lacked myelin completely

and the remainder had thin myelin sheaths (hypomyelination). Complete counts of axons and Schwann cell nuclei were obtained from electron microscope montages of whole transverse sections taken from the mid-portion of the 10 to 15 mm long L4 ventral roots of four adult control and Tr mice¹⁵. The average number ($\pm$ s.e.) of axons in controls (914 ± 17) and Tr mice (856 ± 55) was similar. Schwann cell nuclei were of equal size in both groups of animals, but there was a 10-fold increase in the number of Schwann cell nuclei in L4 ventral roots of Tr mice compared with controls (266 ± 24 and 22 ± 4 respectively). Although this hypercellularity in Tr nerves could be due to a chronic demyelinating process^{16,17}, it may also be secondary to a developmental failure of myelination: the length of Schwann cell territories in teased fibres of Tr mice of all ages studied has been found to be less than normal (unpublished work). Presumably because of the interdependence of myelin thickness and the length of Schwann cell territories¹⁸, both the radial and the longitudinal extension of these cells may be similarly deficient in Tr mice nerves; as a result, axonal ensheathment in fibres which are hypomyelinated or lack myelin must only be possible through a neonatal increase in Schwann cell number. Finally, there were no inflammatory cells or macrophages in Tr sciatic nerves or roots, and neither myelin debris, an indicator of active demyelination, nor concentrically arranged Schwann cell processes (onion bulbs)^{16,17} were prominent features in the Tr nerves we have examined.

Because these quantitative and morphological findings indicated Schwann cell abnormalities and no axonal loss in Tr neuropathy, interactions between axons and Schwann cells were investigated in sciatic nerve grafts. In 26 mice, approximately 6 week old, a 5 mm segment of the sciatic nerve was removed from a donor mouse and immediately grafted between the cut ends of recipient sciatic nerves at the mid thigh level. The graft was secured to host nerve

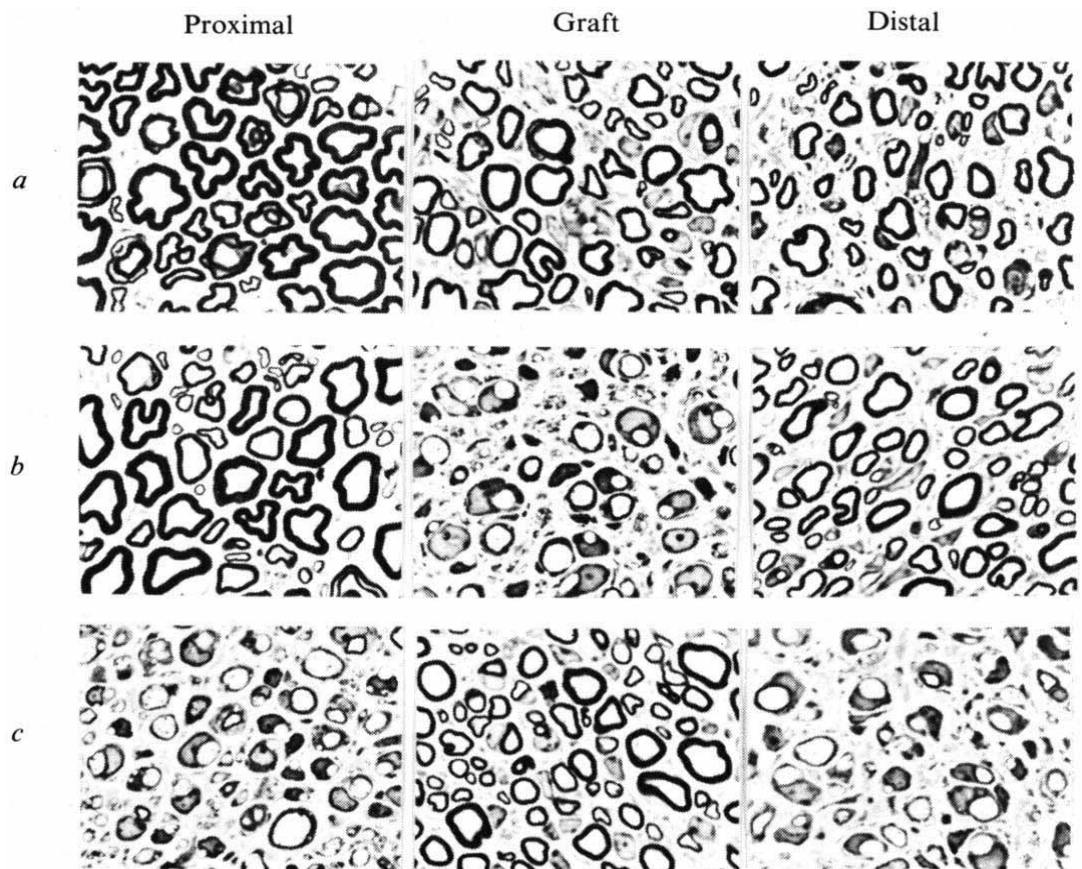


Fig. 1 Phase micrographs of sciatic nerves at three different cross-sectional levels 2 months after grafting. Sections were taken approximately 3 mm from the graft (proximal stump), the mid-portion of the graft, and 3 mm distal to the graft (distal stump). *a*, Normal-to-normal grafts. Grafted segments and distal stumps contain numerous regenerated myelinated fibres which closely resemble those in the proximal stump. *b*, Trembler-to-normal grafts. Proximal and distal stumps (recipient nerves) appear normal, while fibres within the graft either lack myelin or are hypomyelinated. *c*, Normal-to-Trembler grafts. Proximal and distal stumps of host nerves show many fibres with little or no myelin but the morphology of fibres in the graft resembles that of normal regenerated nerves.

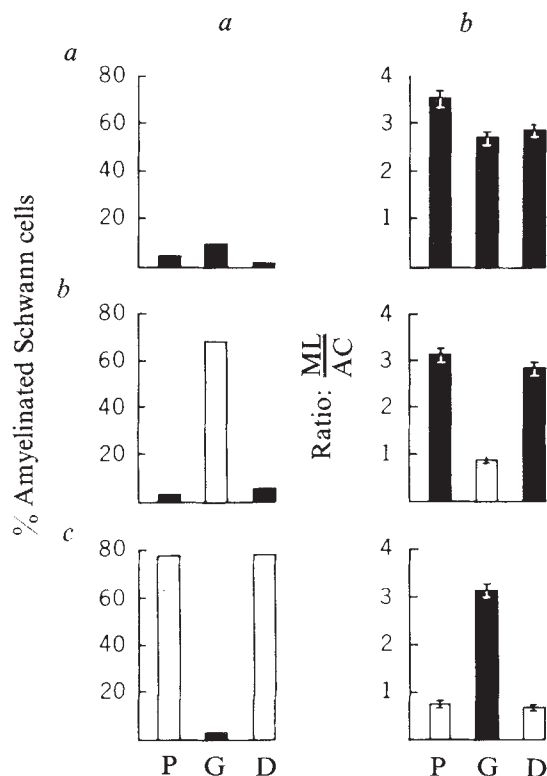


Fig. 2 Proportions of nerve fibres with Schwann cells containing no myelin (a) and ratios of the number of myelin lamellae to axonal circumference (ML/AC) in myelinated fibres (b) from cross sections of proximal (P), graft (G) and distal (D) segments from sciatic nerves 2 months after grafting. Solid bars represent data from normal nerve segments; open bars symbolise values in segments from Trembler nerves. The percentage of fibres with no myelin, calculated at each level for an average of 230 axons greater than 1 μ m in diameter, is expressed as mean $\pm$ s.e.m. The ML/AC was measured in 100 fibres at each level from electron micrographs at a final magnification of $\times 9,100$. In N-N-N nerves, fewer than 10% of regenerated fibres at level G and D lack myelin, and ML/AC ratios are normal²⁰. In normal regenerating nerves, fibres with no myelin probably represent premyelinated fibres as well as nodes of Ranvier, increased in number because of the shortening of Schwann cell territories along regenerated fibres²¹. The proportions of fibres with Schwann cells that contain no myelin and the ML/AC ratios for the proximal and distal segments are similar for N-T-N and N-N-N nerves. In the grafted segments of N-T-N nerves, however, the proportion of fibres with no myelin is increased sevenfold and ML/AC ratios are only one-third those of normal grafts, indicating that myelin sheaths remain inappropriately thin in relation to axonal diameters (hypomyelination). These findings for N-T-N experiments are reversed in the T-N-T experiments: non-myelin containing Schwann cell percentages and ML/AC ratios are normal in the graft but values in the regenerated distal stump resemble those of the Tr recipient proximal stump.

stumps by 10-0 nylon sutures placed through the epineurium. Three different combinations of nerves were prepared:

- Normal-to-normal nerve grafts (N-N-N). Nerves from normal C57BL/6J mice were grafted into sciatic nerves of 14 non-littermate animals of the same strain. Thus, in this group of mice, normal axons regenerating from the proximal stump of the host should associate with normal Schwann cells in the graft and eventually grow into the distal nerve stump.

- Trembler-to-normal nerve grafts (N-T-N). Segments of nerve from Tr mice were grafted between the stumps of sciatic nerves in six normal C57BL/6J mice. In this group, axons from normal nerves should regenerate across a graft containing Schwann cells from abnormal Tr nerves.

- Normal-to-Trembler nerve grafts (T-N-T). Segments from C57BL/6J mice were grafted into six Tr sciatic nerves. In these animals, Schwann cells from a normal graft should ensheath Tr mouse axons.

To prevent graft rejection, anti-lymphocytic serum (prepared by injecting rabbits with a suspension of 10^9 living thymocytes from C57BL/6J mice¹⁹) was injected subcutaneously in doses of 0.5 ml twice weekly, beginning on the day of grafting. Two, three and four months after grafting, animals were killed by systemic perfusion of 3% glutaraldehyde and the nerves processed according to standard techniques for quantitative ultrastructural studies⁶. In all experimental groups, no grafts were rejected and axons from the proximal stumps, whether from normal or Tr mice, successfully bridged the graft.

In all experiments, as regenerating axons grew into the grafts, the morphological features of the donor nerve were fully reproduced by the grafted Schwann cells (Figs 1 and 2). The boundaries between normal and abnormal nerve segments were well delineated in these experiments. In N-T-N regenerated nerves, the widespread hypomyelination and absence of myelin, which are the hallmarks of the Tr neuropathy, were duplicated in the grafts and contrasted abruptly with the normal myelin in the recipient nerve stumps. Because Schwann cells proliferate intensely soon after grafting^{8,11}, these findings demonstrate that the manifestations of the gene responsible for the Tr neuropathy are fully expressed by the transplanted Schwann cells and their daughter cells. There was no indication from the T-N-T experiments that axonally mediated or general systemic factors were involved in the pathogenesis of the neuropathy; the Tr abnormality was not induced in normal Schwann cells transplanted into Tr mice.

These experiments have established the feasibility of *in vivo* combinations of axons and Schwann cells from normal and pathological nerves. They have also proved unequivocally that the Tr neuropathy is due to a primary disorder of Schwann cells. Because conditions in the host animal might be expected to modify the behaviour of transplanted Schwann cells, the technique used in the present study should be helpful in elucidating other pathogenic mechanisms; for example, it should be possible to show whether axons or general metabolic factors are responsible for myelin abnormalities in other neuropathies. Observations in these experiments with Tr mice should also be of value in understanding the pathogenesis of the hypomyelination observed in other neuropathies, both in man^{22,23} and experimental animals²⁴.

Finally, our findings provide further proof that Schwann cells in grafts from distal stumps of nerves regenerating in continuity originate from Schwann cells and not by migration from the proximal stumps^{6,8,11}.

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Spontaneous and evoked potentials from dissociated epithelial cells of *Hydra*

ALTHOUGH intracellular recording from excitable cells has a thirty-year history, such recording from coelenterate tissue has only recently been achieved^{1–4}. Mechanical dissociation of previously separated endodermal and ectodermal epithelial layers of *Hydra* produces a preparation permitting intracellular recording from identifiable classes of cells (Fig. 1). Elicited and spontaneous potentials occur in the cells of both epithelial layers. We have recorded small rhythmically occurring pulses from endodermal cells with positive resting potentials; large spikes from ectodermal cells with negative resting potentials, and regularly occurring membrane oscillations from both types of cells.

Cells could be held for up to 3 h, but we generally withdrew the electrode after 20–45 min. Some records only lasted 10 min (see legend of Fig. 2). Cell types were consistently recognisable (Fig. 1) and pulses were obtained from medium-sized endodermal epithelial cells (20–35 μ m), having few vacuoles or enclosures; from smaller endodermal interstitial cells (10–15 μ m), and from large (>30 μ m) ectodermal epithelial cells. Of the cells penetrated only about 5% produced pulses.

Resting potentials in all solutions were positive for almost all endodermal epithelial and interstitial cells (Fig. 2). These ranged from +3 mV to +25 mV, with the majority between +7 mV and +12 mV. Ectodermal cells had negative resting potentials of –2 mV to –25 mV. The five nerve cells penetrated gave resting potentials of –5 mV to –7 mV. During the usual 4–6 h recording session, all intact cells had resting potentials. Whenever successive penetrations yielded no resting potentials, the cells were judged dead and so discarded.

To compare our resting potentials with those in intact animals, intracellular recordings from endodermal and ectodermal cells of intact *Hydra* were undertaken. Animals were immobilised by prior colchicine treatment^{9,10} and turned inside out¹¹ for endodermal cell recordings. Recordings were done in *Hydra* culture solution¹²; endodermal resting potentials ranged between +15 mV and +20 mV (seven cells); ectodermal resting potentials between –2 mV and –8 mV (eight cells).

Resting potentials of dissociated endodermal cells could be driven to negative values by increasing the sodium concentration above 18 mM. However, the cells produced numerous blebs (presumably a result of osmotic stress) and died within an hour or so. Varying the KCl concentration

from 0.2 to 3 mM produced no apparent change in resting potentials; but raising the molarity above 3 mM KCl resulted in rapid cell death.

We have tentatively identified three categories of electrical activity: Group 1 pulses, reminiscent of typical junction potentials and of epithelial pulses in *Nanomia*¹; Group 2 activity representing apparent membrane oscillations, and Group 3 potentials similar to typical action potentials and to epithelial potentials in *Hippopodius*⁴. The

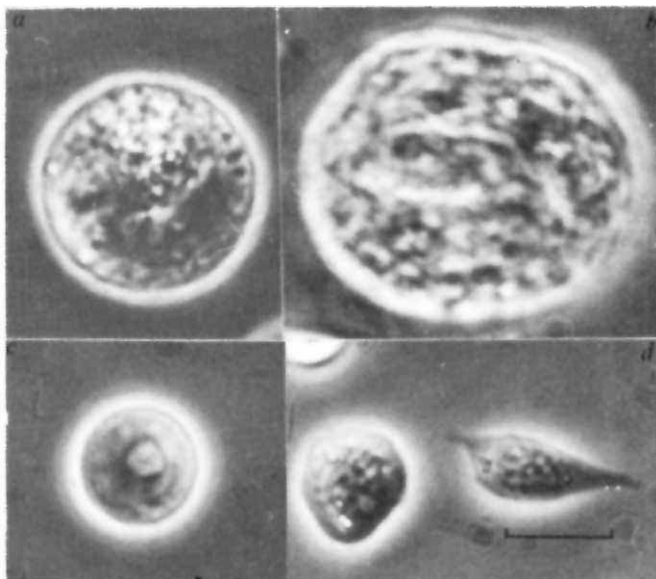


Fig. 1 Dissociated cells. *a*, Endodermal epithelial cell. *b*, Ectodermal epithelial cell containing a glutinant. *c*, Interstitial cell. *d*, Bipolar nerve cell and an interstitial cell. Scales indicate 10 μ m. Preparations of dissociated cells from either or both epithelia of *Hydra attenuata* Pall. are made using methods modified from those of Papenfuss and Bodenham⁵ and Gierer *et al.*⁶ Endodermal epithelio-muscular cells are selectively collected by transecting whole animals at the peduncle, discarding the digestive-cell-bearing upper portions and pipetting the endodermal cells free from the remaining peduncle. Ectodermal cells are gathered by stripping the ectoderm free of endodermal cells in this way and then dissociating the remaining ectodermal shell by gentle pipetting in a centrifuge tube in the recording solution. After pipetting, the cells are filtered through a No. 63 Nitex cloth for additional dissociation and debris removal. Cells are collected by centrifugation for 5 s at top speed, followed by 3 s at medium speed in a clinical centrifuge. They are then pipetted on to a collagen-covered recording dish on the stage of a Zeiss phase contrast inverted microscope and maintained at a temperature of 20 °C by means of a cooling unit in the microscope stage. Preparations were viewed under phase contrast optics (346 $\times$) during recording and both the impaled cell and the recording electrode remained visible throughout.

most striking characteristics in all three categories is the variability of the pulses, both in amplitude and time course, even within a particular sequence of pulses in the same cell.

● Group 1 potentials were recorded from single apparently isolated endodermal epithelial and interstitial cells and from cells in cell aggregates (Fig. 2). They often occurred as regular, rhythmic membrane depolarisations having relatively fast rise times (150 ms–2000 ms) and long decay periods (Time constants ranged between 500 ms and 2–4 s.) Amplitudes of the longer lasting pulses were between +2 mV and +5 mV (Fig. 2*a*); those with faster time constants between +0.3 mV and +0.6 mV (Fig. 2*b*).

The larger rhythmic pulses occurred at an average rate of 2 min^{–1} (1.74 $\pm$ 0.21 (s.e.) for six cells) (Fig. 2*a*). The smaller pulses often occurred sporadically. They are perhaps best construed as miniature junctional potentials, since both negative-going and positive-going potentials of similar amplitudes and time courses were recorded (Fig. 2*b*).

Other pulses, similar in shape, time course and amplitude occurred as brief responses to stimuli given through the

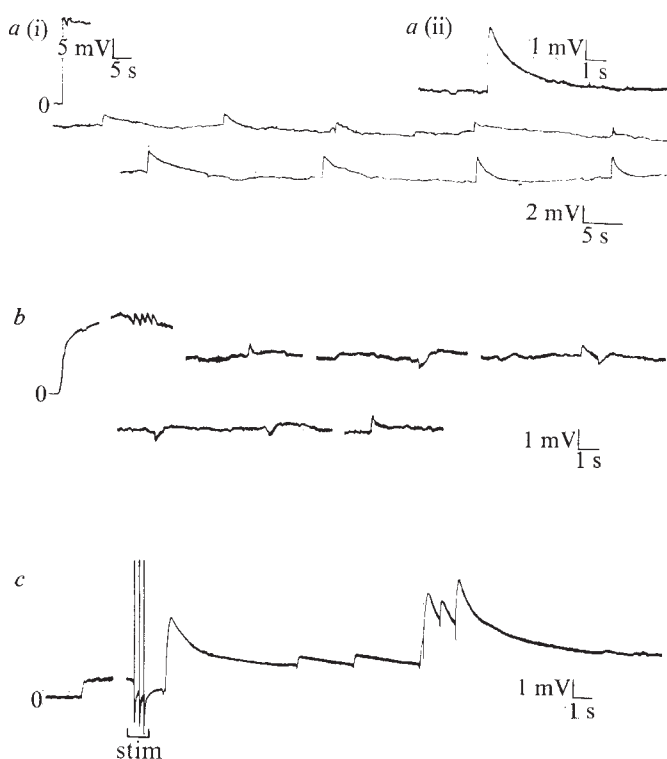


Fig. 2 Group 1 potentials from endodermal cells. Resting potentials are given at the left. Scales at right refer to all preceding pulses. Wave forms in this and all subsequent figures were traced with a pentograph from DC oscillograph records. In *a*(i) spontaneous pulses occur in an isolated cell at a regular rate of about 2 min^{-1} . *a*(ii) is the penultimate pulse enlarged. *b*, Small sporadically occurring spontaneous pulses from an isolated epithelial cell. Both positive and negative-going potentials continued on for 8 min before the electrode was withdrawn. *c*, Positive potentials elicited by a series of stimuli through the recording electrode in an epithelial cell in an aggregate of three cells. The pulses sum or facilitate and culminate finally in a larger, somewhat faster potential. Recordings were made with conventional 3 M KCl filled glass pipettes held by a pair of Jena micromanipulators. Potentials were led to an FET preamplifier with stimulating bridge and simultaneously monitored on a Haer Audio Analyser, a 5103N Tektronix storage oscilloscope and a 7402A Hewlett Packard chart recorder with d.c. amplifiers. Photographs of both cells and oscilloscope traces were taken with Polaroid cameras. A number of solutions were tried as recording media. In different solutions, the salt and sugar concentrations were varied as follows: NaCl from 10 to 15 mM, KCl from 0.2 to 2 mM, CaCl_2 from 5 to 8 mM, MgCl_2 from 0 to 0.3 mM, NaHCO_3 from 0 to 3 mM, EDTA from 0 to 1.4 mM, sugar from 0 to 25 mM. Group 1 recordings were obtained only in sugar-free solutions. Group 2 and 3 recordings in both sugar-containing and sugar-free solutions. All solutions were adjusted to a pH of 6.8 to 7.2 and had an osmolarity of between 60 and 70 mosM, the range of the osmotic concentration of the *Hydra* gut^{7,8}. A solution was judged usable if the cells remained alive in it for 4 h, if the average value of resting potentials remained unchanged for 2 h and if the cells maintained their tonicity for 4 h. Although cells in sugar-containing solutions met the above criteria, they nevertheless eventually swelled and burst even when no glucose was present.

recording electrode. Those in Fig. 2*c* apparently sum or facilitate. They may indeed have resulted from antidromic stimulation to a nerve impinging on the cell.

Whether any of these decrementing pulses are endogenous or a result of nerve activity is not clear, although many were recorded from cells appearing to be nerve free. We may speculate, however, that since the frequency of the regular pulses falls within the range of the extracellularly recorded endodermal rhythmic potentials^{11,13,14} of Passano and McCullough, they are associated with that pacemaker system.

● **Group 2 potentials.** A series of wave-like membrane oscillations were frequently encountered in endodermal epithelial cells and in some ectodermal cells (Figs 3 and 4).

In endodermal preparations, these were often preceded or accompanied by rapid negative-going potentials, occurring either spontaneously or in response to stimuli given through the recording electrode. The negative transients varied from -0.2 mV to -2 mV and usually disappeared after a while. In one case (Fig. 3*b*) they continued to occur in unison with their concomitant waves for some time; both finally stopping spontaneously. Their significance is not known, although in one recording there was an increase in wave frequency after a succession of these spikes.

These wave-like oscillations may represent a series of junctional potentials as described for other systems. In one preparation (Fig. 3) which had been allowed to dark adapt for 10 min and which was then suddenly illuminated through the condenser, the frequency of waves increased from a rate of 32 min^{-1} in the half minute before the light stimulus to 52 min^{-1} in the half-minute after the shock. Since the recording dish was on a microscope cooling stage, and the response to sudden illumination instantaneous, it is unlikely that this was actually a heat response. An increase in pulse rate similarly occurs in the extracellularly recorded rhythmic potential system when the bases of intact *Hydra* are stimulated with light¹⁴. It is quite possible, therefore, that this response may in fact be due to nerve activity.

Alternatively these oscillations may be a function of the membrane itself, since they can occur when spikes from a previously spiking ectodermal cell fail; the oscillations apparently replacing the spikes (Fig. 4). Waves in ectodermal cells also seem capable of developing into spikes (see below).

● **Group 3 potentials** were large, rapid spikes of $+35 \text{ mV}$ to $+200 \text{ mV}$ which could occur individually or in bursts (Fig. 4*a*). These arose spontaneously from cells, approximately 1.5 times the size of medium epithelial cells, which we classified as ectodermal epithelial cells (Fig. 1*b*).

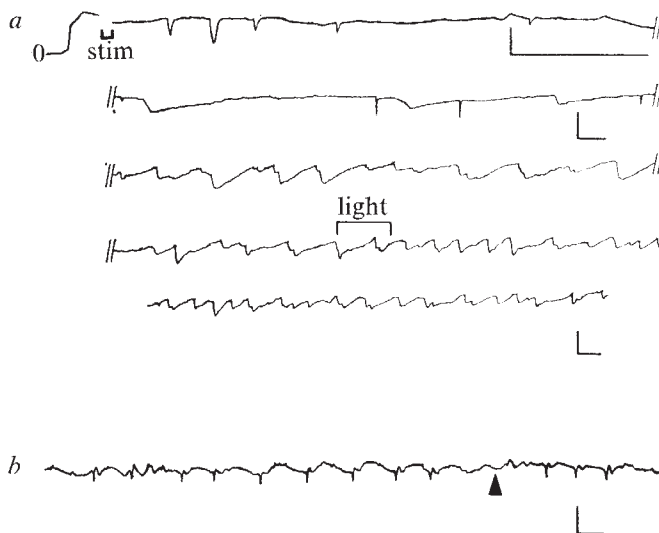


Fig. 3 Group 2 potentials from endodermal cells. Resting potential is on the left. Scales at right refer to preceding pulses (vertical axis equals 1 mV, horizontal axis equals 1 s). *a*, Rapid negative-going pulses elicited by stimuli through the recording electrode, followed by spontaneous oscillations of the membrane which increased in frequency after the first 5 min of recording and then slowed down again. A light shock given 53 min after penetration causes an increase in the oscillatory frequency in the same cell. Deletions from the record greater than 10 min are indicated by slashes. The absence of a slash indicates that the record is continuous. *b*, Spontaneous negative potentials accompanying membrane oscillations in another isolated epithelial cell. Arrow indicates an instance where the wave and spike occurred out of phase.

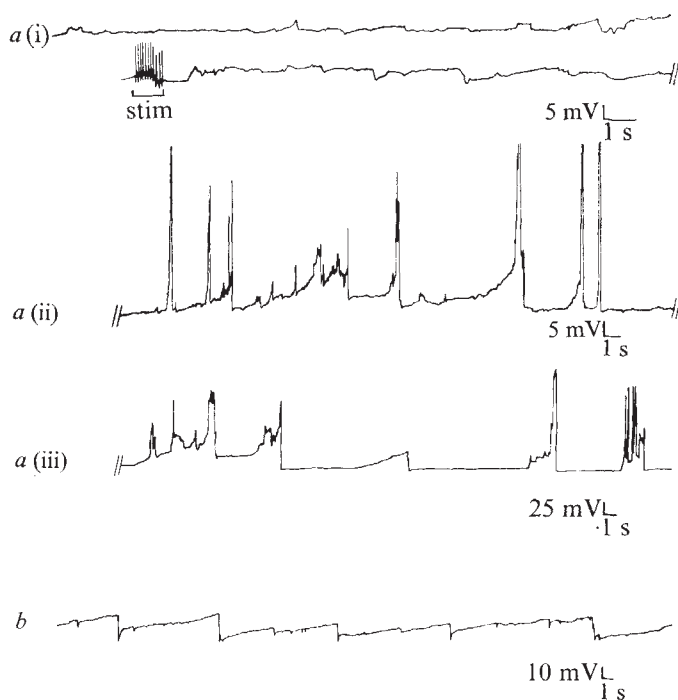


Fig. 4 Group 3 potentials from ectodermal cells having negative resting potentials. *a*(i), Spontaneously occurring membrane oscillations which increase in regularity in response to stimuli through the recording electrode. *a*(ii), *a*(iii), Large spikes occurring singly or in bursts in the same cell. In *a*(iii) the membrane does not always reach threshold. Deletions from the record greater than 8 min are indicated by slashes. The absence of a slash indicates that the record is continuous. *b*, Oscillations of the membrane potential recorded from a cell in an aggregate, which have replaced previously occurring spikes after 2.5 h of spontaneous spiking activity.

The spikes usually arose from gradual depolarisations of the membrane and often had small after-hyperpolarisations. They frequently displayed a distinct rhythmicity (Fig. 4*a*(ii) and *a*(iii)).

As before, these too varied greatly in amplitude and time course. (Durations ranged between 300 ms and 900 ms.) This was so even for a series of potentials in the same burst. Irregularly shaped action potentials of various amplitudes are reported for other systems^{15,16}, as well as for coelenterates².

In one case, spikes arose from successive membrane oscillations which increased in frequency upon stimulation through the recording electrode. These grew into +15 mV depolarisations carrying spikes at their crests; they later became huge individual potentials greater than +200 mV. In another, (Fig. 4*b*) the spikes were replaced after 2.5 h by rhythmic oscillatory waves having small negative-going pulses interspersed among them. (These were perhaps thus inhibitory junctional potentials.) The oscillations were reminiscent of those recorded from endodermal cells (Fig. 3).

Here too, we are not certain whether these pulses are endogenous or the result of nerve activity. But since the membrane potential itself often seemed to become unstable, exhibiting wave-like spontaneous depolarisations comparable to those known for vertebrate smooth muscle¹⁷, it is conceivable that even if this activity were initially induced by nerves, once the cell had become active, it could continue to produce spikes endogenously. A relationship of these spikes to the extracellularly defined contraction burst system¹⁸ is possible, but remains to be investigated.

In summary, then, although it is too early to classify these potentials with certainty, or to assign them to specific

cell types, or to say whether they are endogenously produced by the cells from which they are recorded, it is nonetheless possible to offer tentative suggestions. We would like to propose that, on the basis of their distinct regularity, the rhythmically occurring Group 1 pulses are either junctional potentials which induce the endodermally conducted rhythmic potentials¹³, or themselves underlie these potentials and that the oscillatory Group 2 membrane fluctuations may be a property common to both sorts of excitable epithelial cells. This suggests that *Hydra* epithelial cells possess properties typical of spontaneously active cells whose membranes are capable of prolonged periods of intermittent endogenous depolarisations^{17,19}; even if these depolarisations are initially induced by nerve activity.

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Response of cricothyroid muscles to frequency-modulated sounds in FM bats, *Myotis lucifugus*

BATS of the species *Myotis lucifugus* use short frequency-modulated (FM) ultrasonic pulses for echolocation. The duration, repetition rate and frequency of these pulses vary from time to time depending upon whether the animal is searching for, approaching or actually capturing its prey. The bat emits 2–5 ms pulses at a rate of 10–20 s⁻¹ with the frequency of each pulse sweeping approximately one octave from 80 to 40 kHz during the searching and cruising flight; it produces 0.3–1.0 ms pulses at a rate of 150–250 s⁻¹ with a downward-sweep FM from 40–20 kHz immediately before avoidance of obstacles or capture of a prey^{1,2}. Among the laryngeal muscles of the bat, the cricothyroid muscle (CTM) in particular, is highly developed and is essential in producing the intense orientation pulses^{3,4}. The CTM not only discharges action potentials before the vocalisation^{3,5}, but also during the acoustic stimulus^{6,7}. The latter phenomenon is called the acoustic laryngeal muscle reflex and might serve as a negative feedback to stabilise the performance of the vocalisation system⁶. Comparative studies of the properties of the middle ear and laryngeal muscles revealed significant differences between these two muscles (refs 6 and 7 and P.H.-S.J., J.O., and N. Suga, in preparation). We found that CTM fibres were more sensitive to 4 ms FM signals sweeping downward with a range of 10 or 20 kHz across their best frequencies (BFs) than to pure tones.

Seven *M. lucifugus* were anaesthetised with ether for surgery. The exposed dorsal surface of the skull of a bat was mounted with the flat head of a 1.8 cm long nail with glue and dental cement and the animal was placed ventral side up in a body-sized box made of wire mesh. This box was suspended with rubber bands at its four corners so that it absorbed the body movement from the bat. The head of the animal was immobilised by fixing the shank of the nail into a metal rod with a set screw. The laryngeal muscle was then exposed. After the animals had recovered from the anaesthesia, the action potentials of single CTM fibres were recorded with sharpened and coated tungsten wires from the conscious animals and were displayed on the screen of an oscilloscope. An indifferent electrode of silver wire was placed on the nearby subcutaneous tissue. The electronic instruments used to generate acoustic stimuli were basically the same as described elsewhere^{8,9}. Briefly, tone pulses with duration of 4 ms and 0.5 ms rise-decay time were delivered at a rate of 1.5 s^{-1} from a condenser loudspeaker placed 75 cm in front of the animal. The loudspeaker was calibrated with a Brüel and Kjaer $\frac{1}{4}$ inch microphone placed at the bat's ears and its output was expressed in dB SPL referred to 2×10^{-4} dyne/cm² root mean square. Frequency-modulated signals sweeping at a range of 5, 10, 20 and 30 kHz respectively during the 4 ms duration were generated from a VCG Wavetek (model 186) driven by a sawtooth generator. The sweep range of FM pulses were carefully adjusted so that their centre frequencies were always at the BF of each investigated fibre. The discharge pattern of 52 CTM fibres studied were always tonic-on responding, that is they discharged many action potentials during the stimulus. The shortest latency of this acoustic cricothyroid muscle (CTM) reflex was 6.2 ms which is about 3 ms longer than that of the middle-ear muscle (MEM) reflex. This suggests that acoustic CTM reflex may have a longer neuronal chain than MEMs. The tuning curves of the 52 CTM fibres were of simple triangular shape and were very similar to one another. The best frequencies of these 52 fibres were between 28 and 43 kHz with an average value of 39 ± 1.6 kHz. These tuning curves had sharper slopes both on the high and low frequency sides of the BF and thus had higher Q-10 dB values than that of the MEM fibres. The lowest minimum threshold of the CTMs is about 20 dB higher than the MEMs. When FM signals were delivered, the thresholds of the response were either higher or lower than the thresholds to pure tones depending upon the sweep range and sweep direction of the FM. On the average, a 10 kHz range of downward-sweep FM was 5 dB more effective than a pure tone, but a 20 kHz range of downward-sweep FM was only slightly better. A 10 or 20 kHz range of upward-sweep FM was usually not better than a pure tone. Furthermore, a 5 kHz or 30 kHz range FM signals sweeping either direction was always poorer than a pure tone. In Fig. 1, we present a representative tuning curve of a CTM fibre and its thresholds to FM signals at 10 and 20 kHz frequency sweep ranges. This fibre responded only in a small frequency band between 45 and 29 kHz. Its tuning curve shows a slope of 240 dB per octave and 140 dB per octave at its high and low frequency sides respectively. These slopes are much sharper than those obtained from the MEM fibres^{7,10}. Note that the threshold for 10 kHz range downward-sweep FM is lower than pure tone and the equivalent upward-sweep FM. The threshold difference between the 10 kHz upward and downward sweep FM pulses was as large as 10 dB. The threshold for the 20 kHz range downward-sweep FM was almost the same as for the pure tone of 40 kHz, but the threshold for the equivalent 20 kHz upward-sweep FM was 9 dB less effective. The minimum threshold for this CTM fibre was 54 dB SPL.

Fifty-one of the 52 CTM fibres studied had a threshold higher than 50 dB SPL and a narrow frequency response

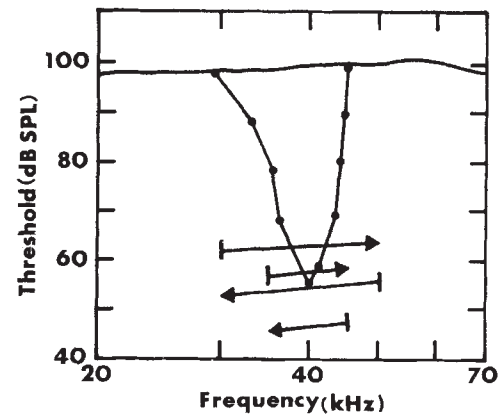


Fig. 1 The excitatory response area (solid line, filled circles) measured by 4-ms tone pulses with 0.5 ms rise and decay times and thresholds for FM tone pulses (arrows) of a representative CTM fibre. The ordinate and abscissa represent the stimulus amplitude in dB SPL (re 0.0002 dyne cm⁻²) and frequency in kHz respectively. The uppermost solid line is a frequency response curve of the condenser loudspeaker used to deliver tone pulses. The arrows represent the directions of frequency sweeps of the 4-ms FM pulses. The different ranges of the FM sweep pulses are represented by the different lengths of the arrows and the thresholds of this CTM fibre for them are shown by the vertical positions of the arrows (see text for the properties of this fibre).

band between 45 and 25 kHz. Because of the frequency content of a bat's orientation sounds^{1,2} and the highly directional sensitivity of its echolocation system¹¹ the acoustic CTM reflex of a bat will generally be evoked only by the sounds emitted by the bat itself or another bat which is flying toward this bat during the approaching or terminal phases. Returning echoes during the terminal phase may also evoke the reflex because their sound amplitudes may be higher than the threshold of this reflex. This speculation is supported by the present results that 10 or 20 kHz range of downward-sweep FM signals mimicking the natural orientation sounds during the final phase of flight were more effective stimuli than the corresponding upward-sweep FM signals or pure tones. An asymmetrical lateral inhibition at the low frequency side of the excitatory response area as that found in the collicular neurones and cortical neurones¹² may also exist in the CTM fibres which causes these fibres to be less sensitive to an upward FM stimulus than to a downward-sweep one.

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Suppression of noradrenaline synthesis in sympathetic nerves by carbidopa, an inhibitor of peripheral dopa decarboxylase

It is generally believed that noradrenaline synthesis in postganglionic sympathetic nerves can be suppressed by drugs which inhibit the activities of tyrosine hydroxylase or dopamine β -oxidase^{1,2}, but not by inhibition of aromatic L-amino acid decarboxylase (AAAD; dopa decarboxylase). We present here evidence that the administration to rats of carbidopa (MK-486; α -methyl-L-dopa-hydrazine), a peripherally acting inhibitor of AAAD³, not only blocks the decarboxylation of exogenous L-dopa (for example, in capillary endothelia within the central nervous system, liver, and kidney^{4,5}), but also suppresses the decarboxylation of endogenous dopa formed from ³H-tyrosine within cardiac sympathetic nerve terminals. As a consequence, the drug inhibits noradrenaline synthesis in this tissue.

Seven separate experiments, all yielding similar observations, were performed, using 122 normotensive male Sprague-Dawley rats (150–200 g) and 60 spontaneously hypertensive (SHR) male Wistar-Okamoto rats (Charles River Laboratories). In each experiment, groups of 4–10 rats received carbidopa (3.2–100 mg kg⁻¹, intraperitoneally, suspended in 0.05 N HCl or 0.9% saline) or its diluent, followed in 60 min by ³H-tyrosine (80–100 μ Ci, in a total volume of 0.1–0.3 ml, intraperitoneally, specific activity 53.3 mCi μ mol⁻¹, New England Nuclear). Animals were decapitated 60 min later and hearts were taken for assay of tyrosine⁶, ³H-tyrosine, ³H-dopa and ³H-catecholamines (including both ³H-dopamine and ³H-noradrenaline)^{7,8}. (In preliminary studies we observed that the rates at which cardiac sympathetic terminals accumulate ³H-dopa and ³H-catecholamines are fairly linear during the 60-min interval used in these studies.)

Table 1 Effect of carbidopa on accumulation of ³H-catechols in cardiac sympathetic nerves

	Control	Carbidopa
Tyrosine (μ g g ⁻¹)	13.1 $\pm$ 0.42	28.9 $\pm$ 1.6*
³ H-tyrosine, specific activity ((d.p.m. μ mol ⁻¹) $\times 10^5$)	7.2 $\pm$ 0.79	12.7 $\pm$ 1.1*
³ H-dopa (d.p.m. per g tissue)	173 $\pm$ 29	380 $\pm$ 29*
³ H-dopa/specific activity of ³ H-tyrosine ((μ mol per g tissue) $\times 10^{-4}$)	8.3 $\pm$ 1.5	9.3 $\pm$ 6.1
³ H-catecholamines (d.p.m. per g tissue)	261 $\pm$ 26	155 $\pm$ 33†
³ H-catecholamines/spec. act. of ³ H-tyrosine ((μ mol per g tissue) $\times 10^{-4}$)	11.5 $\pm$ 1.3	4.7 $\pm$ 1.0*

Groups of 20 male Sprague-Dawley rats received carbidopa (100 mg kg⁻¹, intraperitoneally) or its diluent, and, after 60 min, ³H-tyrosine; the animals were killed 60 min later. Data are given as means $\pm$ s.e.m.

* $P < 0.001$ differs from control values.

† $P < 0.02$ differs from control values.

In all our experiments, carbidopa at least doubled the concentration of tyrosine in cardiac homogenates (Table 1), and caused an even greater increase in cardiac ³H-tyrosine. Thus it increased the specific activity of the ³H-tyrosine present in the hearts (Table 1). For this reason, data on the amounts of ³H-dopa and ³H-catecholamines accumulating in the hearts were expressed both as d.p.m. per g of tissue and as μ mol per g divided by the specific activity of the cardiac ³H-tyrosine. Regardless of the method of data calculation used, the effects of the drug were the same, that is, it increased accumulation of ³H-dopa and decreased that of ³H-catecholamines. In experiments with normotensive rats (Table 1), concentrations of ³H-dopa increased by 120% ($P < 0.001$) and concentrations of ³H-catecholamines decreased by 40% ($P < 0.02$) when expressed as d.p.m. per g

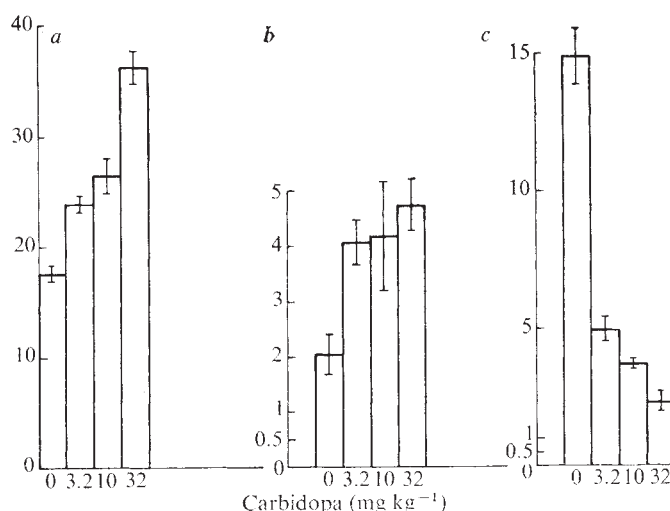


Fig. 1 Effect of carbidopa on the accumulation of ³H-dopa and ³H-catecholamines in hearts of SHR rats. Groups of eight rats were killed 120 min after receiving carbidopa or its diluent intraperitoneally, and 60 min after receiving ³H-tyrosine (80 μ Ci; specific activity 53.3 mCi μ mol⁻¹) intraperitoneally. Data are presented as ³H-dopa (or ³H-catecholamines) divided by the specific activity of cardiac ³H-tyrosine. Vertical bars represent s.e.m. *a*, Tyrosine (μ g g⁻¹); *b*, ³H-dopa/specific activity of ³H-tyrosine (μ mol g⁻¹ $\times 10^{-3}$); *c*, ³H-catecholamines/specific activity of ³H-tyrosine (μ mol g⁻¹ $\times 10^{-4}$).

of tissue; these changes were +12% (not statistically significant) and -59% ($P < 0.001$) when corrected for the specific activity of the ³H-tyrosine.

SHR Wistar-Okamoto rats were found to be highly sensitive to the carbidopa inhibition of AAAD in cardiac sympathetic nerves: a concentration of the drug as low as 3.2 mg kg⁻¹ almost doubled the accumulation of ³H-dopa ($P < 0.001$), and reduced ³H-catecholamine accumulation by 60% ($P < 0.001$) (Fig. 1). Larger doses of carbidopa were even more effective in these animals.

Administration of carbidopa reportedly has no effect on the AAAD in brain neurones of intact animals in the dose range used in our study^{9,10}. In preliminary studies, we also failed to observe any suppression of brain ³H-catecholamine synthesis (d.p.m. per g of tissue) in rats given ³H-tyrosine and carbidopa. Thus, as far as we know, carbidopa may constitute the first clinically useful agent that suppresses noradrenaline synthesis in sympathetic neurones without significantly modifying brain catecholamine synthesis. Its effect on sympathetic nerves might underlie the ability of carbidopa to potentiate the antihypertensive actions of chemically diverse agents in spontaneously hypertensive rats¹¹. Carbidopa might have value in the therapy of diseases involving peripheral catecholamines (or serotonin) in which the physician does not wish to modify brain function.

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Dopamine is a monoamine oxidase B substrate in man

DURING the past decade, the central neurotransmitter, dopamine (DA), has been much studied for it is known to be involved in certain human disease states, Parkinsonism¹ preeminently, and rather more inferentially, schizophrenia². DA is metabolised by monoamine oxidase (MAO)³, which catalyses the oxidative deamination of a wide range of monoamines^{4,5}, including the neurotransmitters noradrenaline (NA) and 5-hydroxytryptamine (5-HT), and other amines such as tyramine, tryptamine and phenylethylamine (PEA). On the basis of animal studies with the inhibitors, clorgyline⁶, and later deprenil⁷, MAO has been classified into two types, A and B. By definition, type A is selectively inhibited by clorgyline and type B by deprenil. In general, type A acts on 5-HT⁸ while type B prefers PEA⁹. In the rat,

Table 1 MAO activity of human platelets with PEA and DA as substrates

Subject	PEA	DA	DA:PEA
1	2.9	3.6	1.2
2	5.7	6.1	1.1
3	16.7	15.4	0.9
4	11.0	14.4	1.3
5	9.8	4.8	1.0
6	4.4	4.4	1.0
7	9.2	9.0	1.0
8	2.2	1.8	0.8
9	11.4	14.2	1.2
10	9.3	8.9	0.9
11	8.8	8.9	1.0
12	14.9	14.3	1.0
13	10.2	10.5	1.0

Activities are expressed as nmol of product formed per mg of protein per 30 min at 37 °C.

DA is preferentially deaminated by MAOA¹⁰⁻¹¹. It thus seemed paradoxical that, in the treatment of Parkinsonism, addition of the MAOB inhibitor, deprenil, to a DA-generating drug combination should produce further therapeutic benefit¹². In this report, we reconcile these apparently conflicting observations by demonstrating that, as far as DA oxidation is concerned, man may be different from rat: in two sites we have investigated, platelet and brain, DA is preferentially deaminated by an enzyme with the characteristics of MAOB.

One way to trace the number and specificity of independent catalytic sites of MAO, which seem to vary both for

tissue and species, is to do cross-inhibition experiments: White and Wu¹³ used a wide range of substrates with human brain MAO and found extensive cross inhibition, apart from with PEA and 5-HT where there was none. Another approach, which we have used, is to see how activity varies when different substrates are used, whether with the same tissue from different individuals or with samples taken from different regions of the same individual. If only one catalytic centre deaminates a particular group of substrates, the ratio of activities should be constant.

We assayed MAO radiometrically by a cation exchange resin method for PEA¹⁴, and an extraction procedure for DA and 5-HT¹⁵. The only modifications of existing methods were of scale: 100 µl of buffer was used with 20 µl of homogenate and 20 µl of substrate. The specific activities of the substrates were also increased fivefold and the final concentrations of DA or 5-HT in the incubation mixture was 150 µM, and of PEA, 50 µM. Incubations were carried out for 30 min at 37 °C and blanks with substrate but no enzyme and with boiled enzyme were incubated with each batch. The reaction was shown to proceed linearly for at least 30 min. Serial dilutions of clorgyline and deprenil in water were freshly prepared: 20 µl of each was added to 100 µl of buffer and 20 µl of enzyme suspension and left at room temperature for 30 min before starting the reaction with ¹⁴C-DA. Platelets were collected from 10-ml venous blood samples collected into 10 ml of 2% EDTA in normal saline¹⁶. Normal human brains, obtained up to 48 h after death, were dissected, and separate regions were stored at -40 °C. Mitochondria and synaptosomes were prepared by differential centrifugation¹⁶.

Human platelets have a type B enzyme which actively deaminates PEA¹⁷. This enzyme has low activity towards 5-HT and is inhibited by deprenil rather than clorgyline. Table 1 shows the activity of platelet MAO towards PEA and DA in samples chosen with widely different PEA-oxidising ability from different individuals. The PEA:DA ratio is constant whether absolute activities are high or low and the two activities are strongly correlated ($r=0.96$). This indicates that the catalytic activities are controlled together and are probably located on the same molecule. The platelet enzyme is as active with DA as with PEA as substrate.

Table 2 shows the results of an experiment in which PEA, 5-HT and DA were used as substrates for MAO from several regions of a single human brain. Here the PEA:5-HT ratio varied greatly from 16 in hypothalamic synaptosomes to 0.7 in substantia-nigral mitochondria, providing support for the existence of independent catalytic sites for 5-HT and PEA. There is clearly DA-oxidising activity in the regions of low 5-HT activity and in this brain it parallels that of PEA. In this brain there was a low 5HT:PEA ratio in the synaptosomal fraction.

We next examined the effects of clorgyline and deprenil

Table 2 MOA MAO activities in different regions of human brain with PEA, 5-HT and DA as substrates

Region	PEA	5-HT	DA	DA:PEA	PEA:5-HT	DA:5-HT
Synaptosomes						
Thalamus	25	9.1	31	1.2	2.7	3.4
Hypothalamus	72	4.5	60	0.8	16.0	13.0
Frontal cortex	13	4.4	11	0.8	3.0	2.5
Substantia nigra	49	29.0	55	1.1	1.7	1.9
Caudate	22	5.6	25	1.1	4.0	4.5
Mitochondria						
Thalamus	92	55	98	1.1	1.7	1.8
Hypothalamus	104	113	82	0.8	0.9	0.7
Frontal cortex	49	52	48	1.0	0.9	0.9
Substantia nigra	94	136	101	1.1	0.7	0.7
Caudate	71	44	73	1.0	1.6	1.6
Nucleus accumbens	75	35	67	0.9	2.1	1.9

Activities are expressed as nmol of product formed per mg protein per 30 min at 37 °C. Assays were carried out in duplicate.

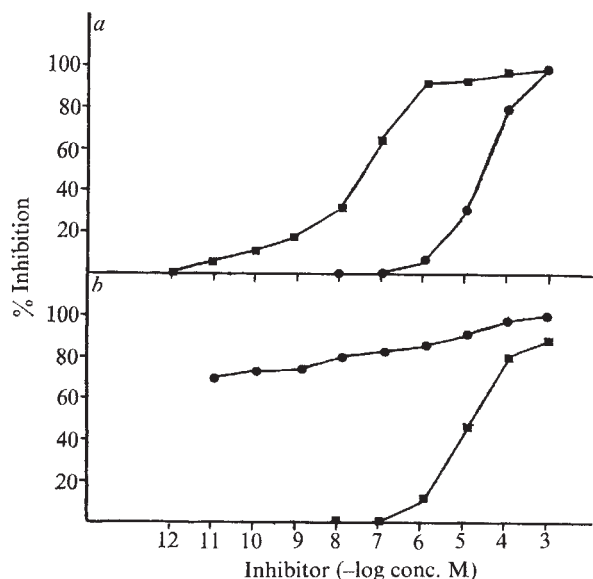


Fig. 1 Percentage inhibition of MAO activity plotted against concentration of deprenil (■) or clorgyline (●) in the incubation mixture, with DA as substrate. *a*, Human caudate mitochondria. Each point is the mean of values obtained using mitochondria from three brains. *b*, Rat brain. Each point is the mean of values obtained with homogenates from two brains.

on the DA-oxidising activity of human caudate mitochondria obtained from three brains. In each case it was selectively inhibited by deprenil (Fig. 1a). We also confirmed the finding¹¹ that the two inhibitors have the reverse effect on rat brain (Fig. 1b). Figure 2 shows the effect of deprenil on MAO from human putamen mitochondria, using 5-HT, PEA and DA as substrates. It demonstrates that the pattern of DA-oxidising activity resembles that of PEA rather than that of 5-HT. At low concentrations of deprenil, PEA and DA are selectively inhibited compared with 5-HT. The DA curve does not exactly follow that for PEA and both extend over several orders of magnitude. It may be that both substrates are oxidised by more than one form of MAO and that some of the DA-oxidising activity is also associated with that of 5-HT.

Although one cannot define the number of forms of MAO catalysing the oxidation of DA in the human

striatum from these results, it is possible to draw the following conclusions. In man, DA oxidation can be independent of 5-HT-oxidising activity, a finding demonstrated most clearly in the platelet. In the human striatum, it is inhibited by smaller concentrations of deprenil than of clorgyline, resembling PEA-oxidising activity rather than that of 5-HT. This DA-oxidising mechanism seems to differ from that of rat brain although this tissue is inhomogeneous, an area with anomalous properties having been detected in the circumventricular region¹⁸. Other anomalous findings in rat¹⁹ and beef²⁰ heart make the conclusion inescapable that MAO is more complex than the simple A/B classification suggests.

Whether the human enzyme shows similar variations from tissue to tissue awaits further investigation. As implied earlier, however, we have shown that the striatal enzyme has properties such that the addition of deprenil to a central dopamine-generating drug regimen¹² now seems to be a rational treatment of Parkinsonism.

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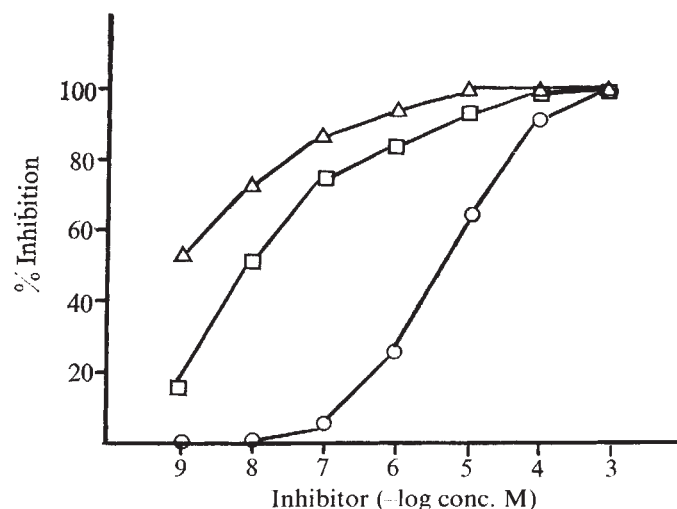


Fig. 2 Percentage inhibition of human putamen mitochondrial MAO activity plotted against concentration of deprenil in the incubation mixture with 5-HT (○), DA (□) and PEA (△) as substrates. Each point is the mean of values obtained by using mitochondria from two brains.

Concanavalin A as a carrier of daunomycin

ONE possible approach for increasing the effectiveness of anti-tumour drugs would be to find methods of altering their distribution in the body to increase their local concentration at the tumour cell sites. Ghose *et al.* have reported the suppression of Ehrlich ascites carcinoma in mice after administration of chlorambucil allegedly bound to anti-tumour antibodies. This immunochemical approach to cancer has great potential, but the production of highly specific antibodies suitable for clinical use may prove to be a major practical problem.

Concanavalin A agglutinates and kills tumour cells *in vitro* under conditions where it has no such effect on normal cells. Administered to mice it kills KHT fibrosarcoma tumour cells and, less markedly, AKR lymphoma cells at doses where it has no effect on numbers of normal hematopoietic colony forming cells². The striking selectivity of con A prompted us to explore the possibility of using the drug as a carrier of daunomycin instead of specific anti-tumour antibody. We describe here the preparation of the conjugate and its striking anti-tumour activity.

Periodate oxidation of daunomycin was performed to cleave the bond between C-3 and C-4 of the sugar moiety, producing carbonyl groups capable of reacting with NH₂ groups on the con A and the resulting Schiff base linkages were reduced with NaBH₄ to form a peptide bond. Daunomycin 40 mg ml⁻¹ in 1 ml of phosphate-buffered saline (PBS) was mixed with a slight molar excess of 0.1 mM NaIO₄ and incubated for 1 h at room temperature in the dark. Glycerol was then added to a final concentration of 0.05 M to consume excess periodate. The solution of oxidised drug was mixed with 1 ml of con A 15 mg ml⁻¹ in 0.15 M potassium phosphate buffer (pH 9.5) and incubated for 1 h at room temperature. NaBH₄ was then added to a final concentration of 0.3 mg ml⁻¹, and the reaction was allowed to proceed for 1 h at 37 °C. Free and bound drugs were separated by gel filtration chromatography, using Sephadex G-100. The degree of substitution was estimated by the drug absorbance at 495 nm and protein concentration was assessed by optical density at 280 nm. The extent of substitution was 2 to 3 mol daunomycin per mol con A. Concanavalin A-daunomycin conjugate was dialysed against PBS for several hours and

Ehrlich ascites carcinoma cells intraperitoneally. After 24 h the mice received the first of five daily injections of the conjugate and control preparations. Table 1 and 2 show the results of such experiments. The greatest increase in survival was obtained when the conjugate was given and Ehrlich ascites carcinoma cells were more sensitive to the conjugate than L₁₂₁₀ cells.

One volume of tumour cell suspensions (1×10^7 cells per ml of PBS) were mixed with 1 volume of concanavalin A solution (375 µg per ml of PBS). After manual shaking, the mixtures were incubated at room temperature for 1 h and the cell aggregates consisting of more than three cells were counted. The aggregated cells of Ehrlich ascites carcinoma and L₁₂₁₀ were 36 and 10 per 200 cells respectively. There may exist some relationship between cell agglutinability by con A *in vitro* and sensitivity to the conjugate *in vivo* although the precise mechanism is not known.

Con A is a potent mitogen for mouse lymphocytes *in vitro*, therefore the conjugate may be toxic for mouse lymphocytes. Steroid hormone inhibits T cell response to mitogen. Intracutaneous injection of con A induces the haemorrhagic Arthus-like lesions⁴ which can be inhibited with steroid hormone. Hydrocortisone was given simultaneously with the conjugate to inhibit lymphocyte damage and Arthus-like lesions and did not inhibit the antitumour activity of the conjugate (Tables 1 and 2).

The con A-daunomycin conjugate may be sufficiently toxic and selective in its effect to be potentially useful in *in vivo* therapeutic studies.

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¹ Ghose, T., Path, M. R. C., and Nigam, S. P., *Cancer*, **29**, 1398-1400 (1972).

² Lin, H., Bruce, W. R., and Walcroft, M. J., *Cancer Chemother. Rep.*, **59**, 319-326 (1975).

³ Hurwitz, E., Levy, R., Maron, R., Wilchek, M., Arnon, R., and Sela, M., *Cancer Res.*, **35**, 1175-1181 (1975).

⁴ Kind, L. S., and Petersen, W. A., *Science*, **160**, 312-313 (1968).

Table 1 Survival of BDF₁ mice bearing L₁₂₁₀ cells

Daily treatment (i.p.) (days 1-5)	Mean survival time (d)
Control	7.0
Daunomycin (0.4 mg kg ⁻¹)	7.4
Con A (8 mg kg ⁻¹)	7.2
Con A (8 mg kg ⁻¹)	
+	7.2
Daunomycin (0.4 mg kg ⁻¹)	
Con A-daunomycin (8 mg kg ⁻¹)	11.4
Con A-daunomycin (8 mg kg ⁻¹)	
+	11.2
Hydrocortisone (2 mg kg ⁻¹)	

in vivo studies were performed within 24 h. Agglutination assays for sheep erythrocytes were performed in a final volume of 250 µl of PBS at a cell concentration of 1×10^8 cells per ml. Con A and con A-daunomycin conjugate equally agglutinated the erythrocytes at the minimum concentration of 2 µg ml⁻¹. SDS polyacrylamide disc gel electrophoresis of the conjugate revealed one protein band with molecular weight about 26,000.

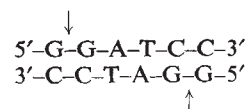
To show the effectiveness of the conjugate as an anti-tumour agent *in vivo*, several groups of five BDF₁ mice were inoculated with 1×10^5 live L₁₂₁₀ cells intraperitoneally and BALB/c mice were inoculated with 2.5×10^6 live

Table 2 Survival of BALB/c mice bearing EAC cells

Daily treatment (i.p.) (days 1-5)	Mean survival time (d)
Control	23.6
Daunomycin (0.1 mg kg ⁻¹)	25.6
Con A (2 mg kg ⁻¹)	27.2
Con A (2 mg kg ⁻¹)	
+	27.8
Daunomycin (0.1 mg kg ⁻¹)	
Con A-daunomycin (2 mg kg ⁻¹)	all alive
Con A-daunomycin (2 mg kg ⁻¹)	
+	all alive
Hydrocortisone (1 mg kg ⁻¹)	

Recognition sequence of specific endonuclease *Bam*HI from *Bacillus amyloliquefaciens* H

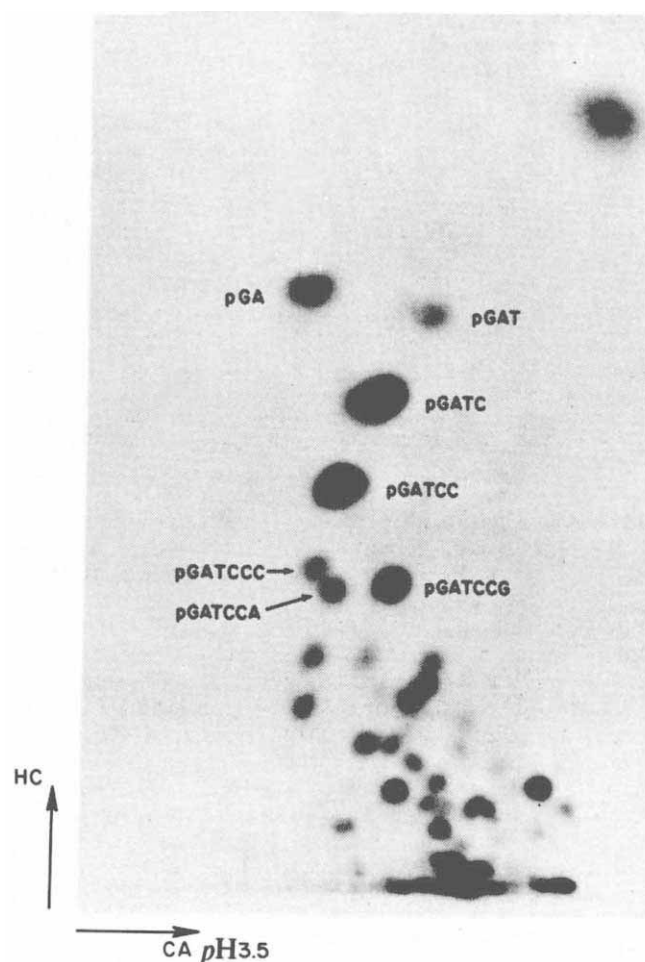
MANY specific endonucleases (restriction endonucleases) have been isolated and recognition sequences have been determined for a number of them¹. The isolation of a new specific endonuclease, *Bam*HI, from *Bacillus amyloliquefaciens* H has recently been described². We have determined the recognition sequence of *Bam*HI and find that it cleaves the two-fold rotationally symmetric sequence



at the positions indicated by the arrows generating fragments with cohesive termini.

The *Bam*HI used for these experiments was purified and used as described previously². This endonuclease gives relatively few fragments from most viral DNAs and, to ensure a statistically significant sampling, parallel experiments were carried out on bacteriophage λ DNA (five sites)², adenovirus (Ad-2) DNA (three sites)² and Φ 80suIII⁺ DNA (eight sites). The experiments described in detail used Ad-2 DNA, which

Fig. 1 Pancreatic DNase digestion products of 5'-terminally labelled *Bam*HI fragments. Ad-2 DNA (10 µg) was incubated in a reaction mix (100 µl) containing 6 mM Tris-HCl pH 7.9, 6 mM MgCl₂, 6 mM 2-mercaptoethanol and 10 units *Bam*HI for 1 h at 37 °C. Alkaline phosphatase (5 µg) was added, the volume increased to 150 µl by adjusting to 50 mM Tris-HCl, pH 8.9, 10 mM MgCl₂ and incubation continued for a further 1 h at 37 °C. Following extraction with phenol (4 × 150 µl), the DNA fragments were precipitated with EtOH and recovered by centrifugation (20 min, 100,000g). The DNA was phosphorylated in a reaction mix (100 µl) containing 50 mM Tris-HCl pH 9.5, 10 mM MgCl₂, 5 mM dithiothreitol, 5% glycerol, 10 µM γ-³²P-ATP (specific activity 1,000 Ci/mmol⁻¹) and 10 units polynucleotide kinase (Biolabs) and incubated for 1 h at 37 °C. Unreacted γ-³²P-ATP was removed by passage over a Sephadex G-50 column run in 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.9, 0.001 M EDTA. Labelled *Bam*HI fragments were recovered from the void volume by precipitation with EtOH (2 volume) and centrifugation (20 min, 100,000g). The DNA was then incubated in a reaction mix (20 µl) containing 0.1 M NaOAc pH 5.0 and pancreatic DNase (5 µg) for 30 min at 37 °C, and lyophilised. This reaction mixture was redissolved in H₂O (3 µl) and fingerprinted using electrophoresis on cellulose acetate at pH 3.5 in the presence of 7 M urea for the first dimension followed by homochromatography⁵ on a thin layer of DEAE-cellulose (1:10) eluting with homomix VI⁶ for the second dimension. The 5'-terminal oligonucleotides were detected by autoradiography, and their sequences deduced from mobility shifts and also as indicated in the text. Oligonucleotides, used for further digestion, were eluted from the thin layer plate in the conventional way⁶.



cannot be labelled with polynucleotide kinase prior to restriction endonuclease cleavage (R. J. R., unpublished results), thus avoiding problems of interpretation due to labelling at the ends of the viral genome. The sequences present at the 5' termini of the *Bam*HI fragments were analysed as follows: Ad-2 DNA was digested with *Bam*HI, the 5'-terminal phosphate was removed by treatment with alkaline phosphatase (an obligatory step) and replaced by a ³²P-phosphate using polynucleotide kinase³ and γ-³²P-ATP⁴. The labelled *Bam*HI fragments were digested with pancreatic DNase and the resulting oligonucleotides fractionated by electrophoresis on cellulose acetate at pH 3.5 in the presence of 7 M urea, followed

Table 1 Identification of the 5'-dinucleotide present after cleavage with *Bam*HI

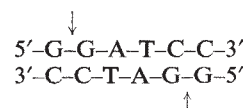
Dinucleotide	R_B (System 1)	R_Y (System 2)
pGA	1.62	0.59
pGC	1.76	0.47
pGG	1.10	0.69
pGT	1.03	0.82
<i>Bam</i> HI product	1.63	0.59

The four standard dinucleotides were prepared by phosphorylating the corresponding dinucleoside monophosphates (Collaborative Research) with polynucleotide kinase (Biolabs) and γ-³²P-ATP as described in the legend to Fig. 1 and purifying the products by electrophoresis on DEAE-cellulose paper in 7% formic acid (System 1). Oligonucleotides from the fingerprint shown in Fig. 1 were incubated in a reaction mix (20 µl) containing 66 mM glycine-NaOH pH 9.6, 6.6 mM MgCl₂, 3.3 mM dithiothreitol and 10 units exonuclease I for 30 min at 37 °C, and spotted on the appropriate paper for fractionation. The oligonucleotide labelled pGA in Fig. 1 was unaffected by this treatment and was identical with the products of digestion of all longer oligonucleotides. System 2 consisted of electrophoresis on Whatman 540 paper at pH 3.5 (ref. 8). R_B refers to the electrophoretic mobility with respect to that of the blue dye xylene cyanol FF⁸ and R_Y to the yellow dye orange G⁸.

by homochromatography⁵ using homomix VI⁶. An autoradiograph of the resulting fingerprint is shown in Fig. 1.

The dinucleotide pGA was identified by (1) digestion with venom phosphodiesterase which liberated only pG; (2) its resistance to the action of exonuclease I⁷; and (3) direct comparison with the four standard dinucleotides pGN (Table 1). The sequences of longer oligonucleotides were inferred from their mobility shifts⁹ and suggest a unique pentanucleotide pGATCC present at the 5' end of each *Bam*HI digestion product. The presence of three of the four possible hexanucleotides pGATCCN indicates that specificity is lost at the sixth position—the absence of the fourth possible sequence being caused by the small number (six) of termini present in the digest used for this experiment. When λ DNA and Φ80suIII⁺ DNA were used in parallel experiments, all four possible hexanucleotides were observed (data not shown).

The simplest interpretation of these results is that *Bam*HI is recognising the hexanucleotide



with the sites of cleavage as indicated by the arrows. This would mean that *Bam*HI fragments possess a 5'-tetranucleotide extension and hence should be a substrate for a DNA polymerase. This prediction was tested by preparing *Bam*HI fragments of Ad-2 DNA and incubating them with avian myeloblastosis virus (AMV) reverse transcriptase in the presence of one α-³²P-deoxynucleoside triphosphate and three unlabelled deoxynucleoside triphosphates. All four possible combinations were used and the products were analysed by nearest-neighbour analysis. The results of these experiments are shown in Table 2

Table 2 Nearest neighbour analysis of nucleotides incorporated into *Bam*HI treated Ad-2 DNA by AMV reverse transcriptase

Labelled substrate	pmol ³² P-dNTP incorporated		Products of micrococcal nuclease and spleen phosphodiesterase digestion†				Sequence deduced (5'→3')
	<i>Bam</i> HI treated Ad-2 DNA*	Ad-2 DNA*	Cp	Ap	Gp	Tp	
α- ³² P-dATP	7.2 (6)	0.21 (0)	18	16	62	4	GpA
α- ³² P-dCTP	6.8 (6)	0.10 (0)	13	12	10	65	TpC
α- ³² P-dGTP	7.8 (6)	0.16 (0)	18	17	52	13	GpG
α- ³² P-TTP	7.1 (6)	0.13 (0)	15	54	18	13	ApT

*Bam*HI fragments of Ad-2 DNA and untreated Ad-2 DNA were used as templates for the incorporation of one α-³²P-deoxynucleoside triphosphate and three unlabelled deoxynucleoside triphosphates with AMV reverse transcriptase. 23 μg (1 pmol Ad-2 DNA) of fragments were used in each experiment using conditions previously described¹⁰. An aliquot of the reaction mixture was precipitated with perchloric acid to determine the total incorporation. The resulting labelled DNA was completely digested with micrococcal nuclease and spleen phosphodiesterase¹¹ and the resulting mono-nucleoside 3'-phosphates analysed by electrophoresis on Whatman 540 paper at pH 3.5. The separated mononucleotides were visualised by radioautography, cut from the paper and quantitated by liquid scintillation counting.

*The number in parenthesis is the theoretical incorporation calculated from the deduced sequence and for the three *Bam*HI sites present in Ad-2 DNA.

†Quantities of 3'-mononucleotides expressed as a percentage of the total mononucleotides detected.

and provide confirmation of the recognition sequence assigned earlier on the basis of mobility shifts.

The data from these experiments involving repair synthesis indicate that there is some heterogeneity at the 3' terminus in contrast to the homogeneity at the 5' terminus. The amount of heterogeneity observed varied considerably from one experiment to another and is most easily interpreted as due to the presence of a contaminating 3'→5' exonuclease (similar to exonuclease III of *Escherichia coli*)¹² present in the preparation of *Bam*HI used for these experiments.

Like *Eco*RI¹³ and *Hind*III¹⁴, *Bam*HI generates fragments bearing cohesive termini. This should prove useful for the production of recombinant DNAs and a vector suitable for *Bam*HI fragments has already been described¹⁵.

Exonuclease I was a gift from R. Wu.

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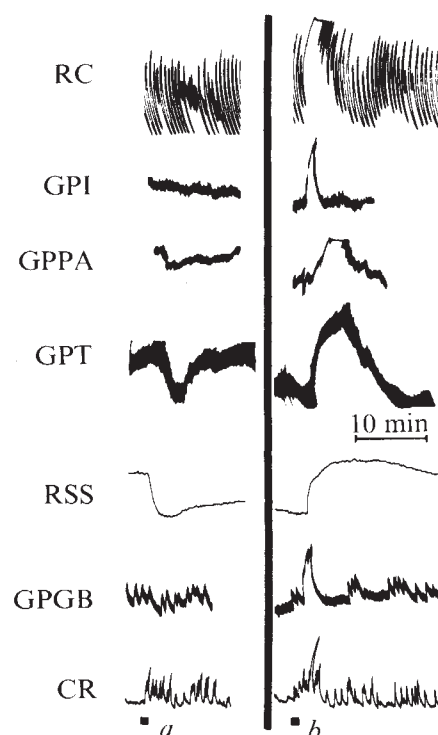
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Relationship of spasmogenic and smooth muscle relaxant peptides from normal lung to other vasoactive compounds

ALTHOUGH abnormal lung tissue, especially certain bronchogenic tumours, may secrete a variety of peptide hormones¹, none has been isolated from normal lung. We have now extracted from normal hog lungs peptide fractions containing at

least two active principles, one of which relaxes and the other contracts non-vascular smooth-muscle organs. Like the recently isolated vasoactive intestinal peptide (VIP)², both pulmonary peptide fractions are systemic vasodilators. The relaxant peptide fraction further resembles VIP in its smooth-muscle effects, and binds with antibodies against this peptide. The spasmogenic peptide fraction contracts a variety of smooth-muscle organs that are also contracted by prostaglandins, bradykinin, angiotensin II, substance P, and the "slow-reacting substance of anaphylaxis". Immunoreactivity attributable to the relaxant, VIP-like peptide, was found in normal lungs of several mammals, including man, and in pulmonary oedema foam.

Fig. 1 Responses of isolated, superfused smooth-muscle organs to 125 μg ml⁻¹ of *a*, relaxant and *b*, spasmogenic lung peptide fractions, infused at 1 ml min⁻¹ (data from two experiments). RC, rat colon; GPI, guinea pig ileum; GPPA, guinea pig pulmonary artery; GPT, guinea pig trachea; RSS, rat stomach strip; GPGB, guinea pig gall bladder; CR, chick rectum. Assay organs had been pretreated with a mixture of pharmacological blockers containing: 10⁻⁷ g ml⁻¹ mepyramine maleate, 2 × 10⁻⁷ g ml⁻¹ methysergide bimalate, 2 × 10⁻⁶ g ml⁻¹ propranolol HCl, 10⁻⁷ g ml⁻¹ phenoxybenzamine HCl, and 10⁻⁷ g ml⁻¹ of hyoscine HBr. Peptide solutions were infused at mark below. Upward movement is contraction, and downward movement relaxation.



The extraction procedures were similar to those used in the isolation of VIP³. Fresh hog lungs were boiled for 10 min, minced and extracted in dilute acetic acid. The filtered extract was treated with alginic acid to adsorb peptides, which were later eluted with HCl, salted out with NaCl and extracted with methanol. During these procedures, repeated washing with lipid solvents ensured the removal of prostaglandins and other lipids. From the methanol extract, peptides were recovered in aqueous solution and then desalted and fractionated on a column of Sephadex G-25, with dilute acetic acid as eluant. The active fractions were chromatographed on a column of carboxymethyl cellulose (CMC), using phosphate buffer (pH 6.4) and a gradient of NaCl. Further purification of the smooth-muscle relaxant peptide fraction was obtained by a second chromatography on CMC, at pH 8, followed by counter-current distribution in a 1:1 mixture of 0.1 M ammonium bicarbonate and *n*-butanol.

During the extraction and purification, test solutions were assayed by their vasodilator action on the systemic circulation of anaesthetised dogs⁴, and by their effects on isolated, superfused smooth-muscle organs⁵. The active peptide fractions induced peripheral vasodilation, evidenced by an increase in femoral blood flow and a fall in aortic blood pressure on infusion into the femoral artery. When tested on smooth-muscle organs, these fractions were found to contain two active principles, having opposite actions. Thus, one fraction contracted, and the other relaxed: stomach strip and colon of rat; trachea, pulmonary artery, ileum and gall bladder of guinea pig; and chick rectum (Fig. 1). These effects were dose-related (Figs 2 and 3), were unaltered by pharmacological blockade of histamine, 5-hydroxytryptamine, adrenergic or cholinergic receptors, but were almost totally abolished by incubation with the proteolytic enzyme pronase (Calbiochem, 20 μ g per mg peptide fraction).

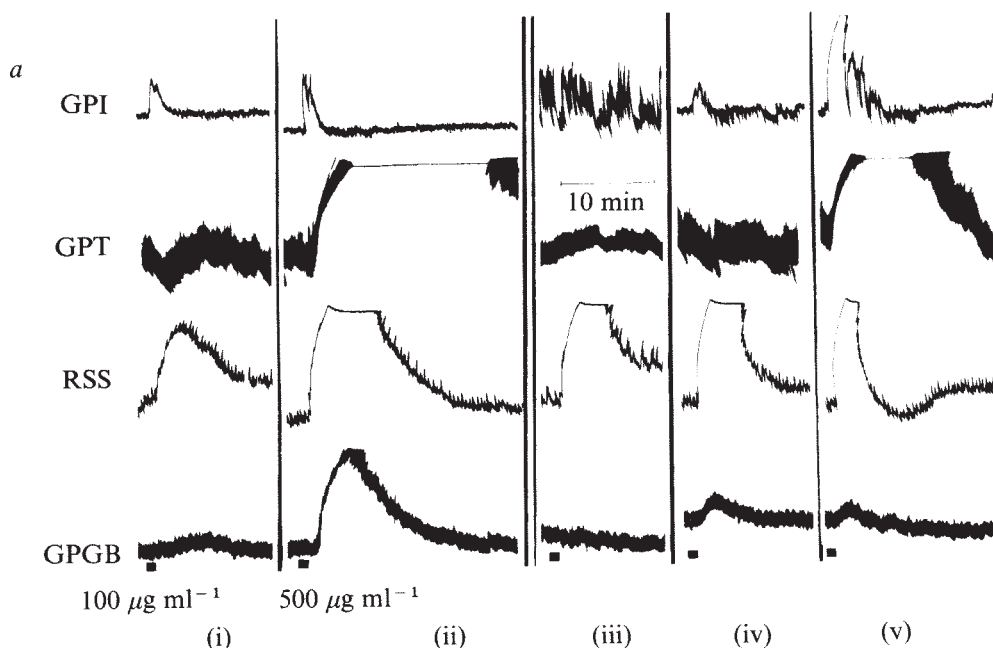
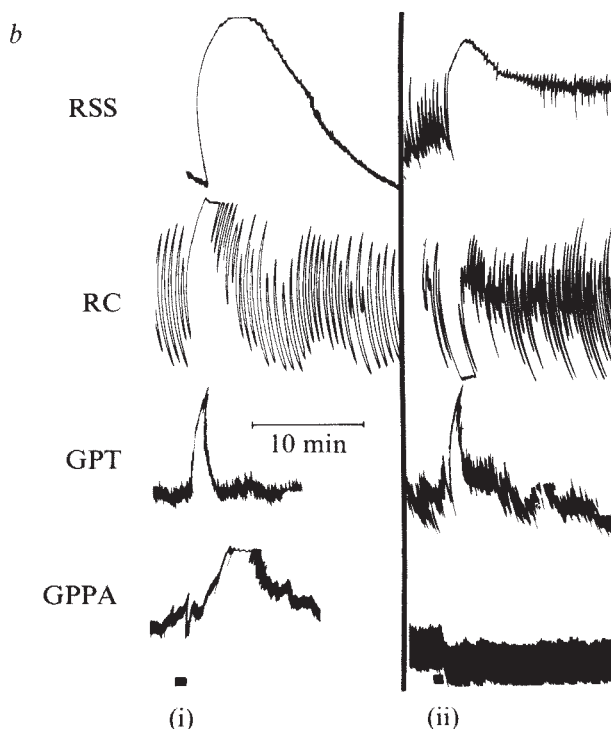


Fig. 2 *a*, Responses of guinea pig ileum (GPI), guinea pig trachea (GPT), rat stomach strip (RSS), and guinea pig gall bladder (GPGB) to two doses (100 and 500 μ g ml⁻¹) of spasmogenic lung peptide fraction (i and ii) and to prostaglandin F_{2 α} (50 ng ml⁻¹) (iii), prostaglandin E₂ (5 ng ml⁻¹) (iv) and substance P (1 μ g ml⁻¹) (v). All agents were dissolved in Krebs solution and infused at 1 ml min⁻¹. *b*, Responses of RSS, rat colon (RC), GPT and guinea pig pulmonary artery (GPPA) to same spasmogenic peptide fraction (125 μ g ml⁻¹) (i) and to bradykinin (50 ng ml⁻¹) (ii).



The smooth-muscle actions of the relaxant lung peptide fraction are similar to those of VIP, which relaxes the above-listed smooth muscle tissues^{6,7}. This lung peptide fraction also competed with labelled VIP in binding to anti-VIP antibodies in a radioimmunoassay that detects 20 pg of the porcine peptide per ml^{8,9}. With progressive purification, culminating in counter-current distribution, this binding capacity increased along with the increase in biological activity and was at least 15% that of pure porcine VIP. The displacement of bound VIP with different dilutions of this peptide paralleled the standard curve for pure VIP. In the same assay, antibodies to VIP showed less than 1:1,000 cross reaction with secretin (GIH Laboratory, Karolinska Institute), glucagon (Eli Lilly), cholecystokinin-pancreozymin (GIH Laboratory), bradykinin (Sandoz), substance P (Beckman), or somatostatin (ovine, Beckman).

The spasmogenic peptide fraction, on the other hand, exhibited negligible (<0.1%) binding capacity to VIP antibodies. The smooth-muscle actions of this peptide fraction resembled those of other vasoactive substances (Fig. 2), but could be distinguished from these by examination of the full spectrum of biological activity. Thus, prostaglandin E₂ in a dose (5 ng ml⁻¹) giving nearly equal contraction of guinea pig ileum, relaxed guinea pig trachea and gave a stronger contraction of rat stomach strip. Prostaglandin F_{2α} (50 ng ml⁻¹) contracted guinea pig trachea but was without effect on ileum and gall bladder. Substance P, in a dose (1 µg ml⁻¹) causing a stronger contraction of ileum, was less active on gall bladder and trachea. The spasmogenic lung peptide could also be distinguished from bradykinin, which relaxed rat colon and a strip of guinea pig pulmonary artery, and from angiotensin II, which is a vasoconstrictor. The incompletely identified "slow-reacting substance of anaphylaxis", was not available to us for comparison. This substance has been detected and assayed in biological solutions and tissue extracts on the basis of contraction of guinea pig ileum or trachea, in the presence of atropine and an antihistaminic¹⁰. These actions alone do not, however, permit a differentiation between the slow-reacting substance and the spasmogenic lung peptide.

Because of the above findings in purified peptide fractions from lung, crude extracts of normal lung from several species (dogs, cats, rabbits, and human subjects undergoing thoracic surgery) were examined for VIP-like immunoreactivity. The extracts, prepared by boiling for 10 min and extracted in acid alcohol, contained the equivalent of up to 2.8 ng immunoreactive VIP per g wet weight. On induction of pulmonary edema in anaesthetised cats (by rapid intravenous infusion of dextran-saline solution) or in isolated, perfused cat lungs (by

raising outflow pressure), oedema fluid and foam contained approximately 300 pg ml⁻¹ of VIP immunoreactivity.

The apparent presence in the lung of a VIP-like peptide may be explained by the embryological derivation of the lung from foregut, or by the presence of neuro-endocrine (APUD) cells, of neural crest origin, in the lung as well as the gastrointestinal tract¹¹. This observation is another example of the occurrence of VIP-like bioactivity and immunoreactivity outside the gastrointestinal tract; related activity has recently been found in high concentrations in the brain and other nervous tissue⁹. The presence of two peptides with opposing actions on tracheo-bronchial and vascular smooth muscle (and other contractile elements in lung¹²) provides a potential mechanism for regulating the distribution of alveolar ventilation and perfusion. The demonstration of one of the vasoactive lung peptides in pulmonary oedema foam suggests that this, and possibly also the other peptide, may be released in clinical settings associated with pulmonary oedema, for example heart failure, "respiratory distress syndromes", oxygen poisoning, and anaphylaxis. The recent finding of increased plasma VIP immunoreactivity in some patients with lung tumours⁸ could have been due to secretion of the VIP-like pulmonary peptide.

Because of the similarities in biological actions between the spasmogenic lung peptide, other vasoactive peptides (bradykinin, angiotensin II and substance P), the prostaglandins, and the "slow-reacting substance of anaphylaxis", the possibility of release of this peptide should be considered whenever spasmogens are released from the lung. The use of multiple bioassay organs should help to distinguish between the different compounds, especially if they are present singly.

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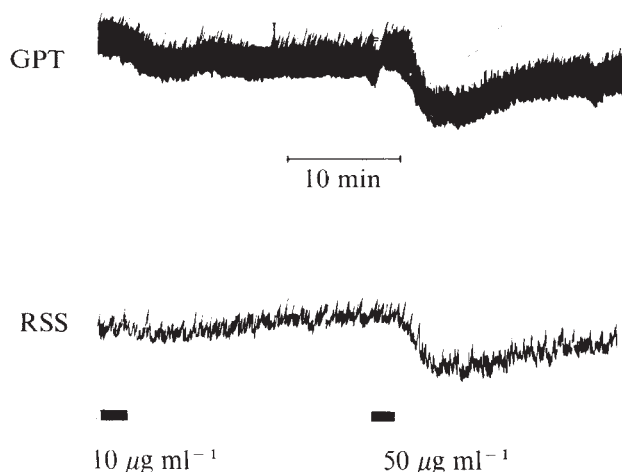
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Fig. 3 Dose-related relaxation of guinea pig trachea (GPT) and rat stomach strip (RSS) in response to infusion of 10 and 50 µg ml⁻¹ of relaxant peptide fraction.



Errata

In "Airborne ice nuclei near an active volcano" by R. C. Schnell and A. C. Delany (264, 535-6) the labels to curve families c and d in Figure 2 were interchanged. The upper family should have been labelled d, the lower c.

In "Suppression of murine lupus erythematosus by Dactinomycin" by A. E. Gabrielsen, A. S. Lubert and C. T. Olsen (264, 439-440) Figures 1 and 2 were interchanged—the captions are correctly placed.

reviews

Historical essays on mycology

C. T. Ingold

Introduction to the History of Mycology. By G. C. Ainsworth. Pp. xi+359. (Cambridge University: Cambridge, London and New York, 1976.) £11.

MYCOLOGISTS have always lacked a comprehensive history of their subject. Many of the older mycologists have read with profit Whetzel's *An Outline of the History of Phytopathology* (1918) and have relished Ramsbottom's *The Expanding Knowledge of Mycology Since Linnaeus* (1941). And a much wider circle has also rejoiced in Large's *The Advance of the Fungi*. But now at last here is a full-scale history of mycology, although, with characteristic modesty, the author presents it as an *Introduction*.

Only one with an encyclopaedic knowledge of the literature on fungi could have produced this work which fittingly comes from the pen of one who can be described as the Dr Johnson of mycology. No other worker in this field can exist without almost daily reference to his *Dictionary of Fungi*.

Dr Ainsworth has sensibly not attempted to keep all sections of the subject in play while proceeding steadily from the earliest times to the present day, but has rather produced a series of historical essays on various aspects of mycology. These form his chapter headings: origin and status of fungi; form and structure; culture and nutrition; sexuality, cytology and genetics; pathogenicity; poisonous, hallucinogenic and allergenic fungi; classification; and finally the organisation of mycology.

P. A. Micheli, born in 1679 and the author of *Nova Plantarum Genera* (1729), is rightly regarded as starting the scientific study of the fungi; as the father of mycology. We learn from the English translation of his epitaph in Florence that he "was much beloved by all the worthy men of his age on account of his wisdom, sweetness of disposition and modesty". A number of figures from his magnum opus are reproduced: the full title-page; his lovely plate of *Clathrus*; his representation of agaric structure and development: his drawings of almost pure cultures of moulds on melon slices; his illustrations of *Aspergillus* and *Botrytis*; and his dynamic figure of a cup-fungus



Mayan mushroom stone (British Museum, London), probably from Guatemala and believed to date from the middle of the first millennium AD—early evidence for a mushroom cult in Central America.

puffing. Also reproduced are Micheli's original drawings of asci, the first ever made, showing clearly that the block-maker did a rather poor job at times in preparing the plates for the printed book.

There is a basic difficulty about a history that extends from the misty past to the present. In the earlier part much must be made from very little: and in the more recent part there is such a wealth of material that the historian is faced with an almost impossible task of selective condensation. It is, of course, easy to criticise this selection from recent history. For example, in connection with mycorrhiza no mention is made of Melin's work and the reader is referred for a recent view on ericaceous mycorrhiza to a 1927 review by Rayner! Again, in the genetics section the early work of Hansen and Smith in 1932 on heterokaryosis does not figure; indeed, the word does not

occur in the index. But any criticisms of this nature are of marginal significance.

The chapter on pathogenicity is particularly rewarding because the author, with his profound knowledge of medical and veterinary mycology, presents a balanced picture in which diseases of plants and of animals (including man) are nicely balanced. Occasionally, perhaps inevitably, a chapter becomes something of a catalogue and this is most noticeable in the chapters on the distribution of fungi and on classification.

Illustrations are a special feature of the book. In all, 106 carefully selected figures—including reproductions of historic drawings; portraits and photographs of eminent mycologists; and facsimile reproductions of pages from books and manuscripts—provide a distinguished atmosphere for the book.

A brief section which many will enjoy deals with hallucinogenic fungi. The Wassons together with Roger Heim are regarded as the founders of ethnomycology.

The final chapter on the history of the organisation of the subject is stimulating. It is concerned with mycological societies, journals and books. Due credit is given to de Bary's textbook. Although over a hundred years old, this great work is still a model that has never been surpassed and is still an inspiration to working mycologists. The same chapter is enlivened by one of Worthington Smith's delightful cartoons of fungal forays a century ago.

Another mycologist writing a history (but it is difficult to think of anyone else who could do it) would certainly achieve a different balance. To some extent the balance reflects Dr Ainsworth's own interests but happily these are exceptionally wide. It is perhaps not a book for the undergraduate student, but it will be read with enormous pleasure by all mycologists with a broad interest in their subject. Every library in an institution where fungi are studied will have to possess a copy and many individual workers will want one of their own. □

C. T. Ingold was Professor of Botany at Birkbeck College, University of London, UK, from 1944 to 1972.

Insect integuments

The Insect Integument. Edited by H. R. Hepburn. Pp. xix+571. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl.165.00. \$63.50.

THIS *Festschrift* is dedicated to A. Glenn Richards, author of the classic *Integument of Arthropods* (1951) and constructor of the first electron microscope ultramicrotome (1942). In a biographical first chapter, M. Rockstein describes the life and work of 'Glenn', including a bibliography. The miscellany of 26 papers naturally contains much that is already well-known. In the space here available I have therefore selected those items whose originality impressed me.

K. M. Rudall presents excellent X-ray diffraction diagrams from cuticles and from *Sagitta* (Chaetognatha) spines. Had one been more fully Miller-indexed, however, it would make the text easier to follow. V. Strout, H. Lipke and T. Geoghegan show by formic acid extraction a narrowing of the range of molecular weights of chitin as the last larval cuticle becomes that of the puparium in flies. This facilitates chitin reorientation at this stage.

In an invigorating chapter, R. A. A. Muzzarelli provides a useful collateral of chemical knowledge from fungal and bacterial research. This includes native chitosan, and the enzymes responsible for its formation from chitin and subsequent degradation. He also reviews chitin—carotenoid linkage in cuticles, and new insecticides acting as inhibitors of chitin synthesis, rightly warning of the danger of their indiscriminate use.

V. B. Wigglesworth emphasises the possible significance of lipids as structural polymers in cuticle. There follows a pioneer attempt (R. H. Hackman) to explain the large number of protein species in cuticles as polymorphism, due to substitution mutations in the second base of genetic code triplets. But the supporting evidence seems to require statistical analysis. S. O. Andersen provides a comprehensive review of cross-linking enzymes both in haemolymph and the cuticle itself. P. Karlson and C. E. Sekeris review the morphogenetic action of ecdysone, augmenting their classical results with recent evidence. P. J. S. Furneaux and A. L. Mackay describe a 90° cross-ply fibril array in a beetle eggshell, the first in insects, as far as I am aware. R. Dennell revives a mechanistic theory for control of chitin orientation, but does not mention existing published evidence against it. H. R. Hepburn and I. Joffe have compiled useful and detailed tables of mechanical properties for chitin and whole cuticle. On the

basis of stress-strain curves and fractography they distinguish cuticles which show plastic fracture, brittle fracture, or a combination of both. M. Locke relates cuticle formation to alternate secretion by plasma membrane plaques and Golgi vesicles.

S. Caveney presents results which may shed light on the unsolved problem of cell morphogenetic gradients. He shows that insect epidermis functions as a syncytium by cell/cell communication via gap junctions. The late J. M. Whitten records the regular orientation of centriole-like structures in the cells of fly feet, and suggests that they may be related to formation of regular hair patterns. E. P. Marks and B. A. Sowa are successfully exploiting *in vitro* cuticle growth methods for investigating blocks to chitin synthesis and effects of insecticides on cuticle formation under carefully controlled conditions. Chapters by H. Nemenz and W. Ebeling both stress the importance of epidermis rather than epicuticle as a water-controlling mechanism in relation to osmoregulation and insecticides respectively.

J. F. V. Vincent compares stress-softening in locust intersegmental membrane and filled rubber composites,

while H. C. Bennet-Clark provides a lucid account of jumping principles and cuticular energy storage mechanisms. F. G. Barth summarises results of photoelastic analysis of strains applied to perspex models of the cuticular parts of arachnid mechanoreceptors. Ablation experiments have pinpointed the specific sense organs predicted to be responsible for the idiothetic sense of spiders. This is the sense of orientation using the experience of previous movement sequences, without using landmarks. H. E. Hinton reviews a wide range of recent work on insect physical colour systems. He records anti-reflection nipples (previously thought to be restricted to corneal surfaces) on the wing scales of the green hairstreak butterfly. W. Kenchington presents an intriguing range of modes of formation of peritrophic membranes. W. Peters summarises recent results on *in vitro* growth of peritrophic membrane, and factors which affect it.

The volume is well produced and will clearly be necessary reading for relevant workers—if their institutions can afford it.

A. C. Neville

Dr Neville is a Reader in Zoology at the University of Bristol, UK.

Atmospheric radiative transfer

Radiative Processes in Meteorology and Climatology. (Developments in Atmospheric Science, Vol. 5.) By G. W. Paltridge. Pp. xvii+318. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl.103; \$39.75.

THE basic atmospheric engine operates between a source which is derived from solar radiation absorbed by the atmosphere, or by the surface, and a sink which is the emission of infrared radiation to space. Radiation processes are, therefore, of fundamental importance to atmospheric study. Further, because of the current concern about the global climate and possible mechanisms through which climate may change, there is a lot of interest in the Earth's radiation budget and the possibility of small alterations which might occur in it, due to changes in the radiation input or output. For instance, variations in the net solar input might occur either due to changes in the sun itself or changes in the atmospheric cloud or dust content; or variations in the emitted radiation from the atmosphere itself might occur for similar reasons.

There are few textbooks available on atmospheric radiative transfer. One of the best of the standard texts written by R. M. Goody in 1964 is now out of print, so that this new text

by Paltridge and Platt is very much to be welcomed. It presents the fundamental theory of radiative transfer, and shows how empirical and theoretical approximations may be applied to the transfer of radiation in the clear atmosphere and in clouds. A lot of very useful up-to-date information is contained in the book about the radiative properties of the surface, of different types of clouds and aerosol, and about atmospheric transmission in different spectral regions. Emphasis is placed on radiative processes in the troposphere; little is said about the high atmosphere.

The book does not set out to be a thorough theoretical treatise. In fact, some of the theoretical presentation is confused (for instance, the presentation of the Curtis-Godson approximation in chapter 7 and its confusion with the pressure scaling of absorber amount). Rather, the book is aimed at providing the methods and means for meteorologists and atmospheric physicists who need to incorporate radiation processes into atmospheric models or work out simple practical atmospheric problems involving radiative transfer. For such people the book will be very valuable indeed and well worth purchasing.

J. T. Houghton

J. T. Houghton is Professor in the Department of Atmospheric Physics at the Clarendon Laboratory, University of Oxford, UK.

North American fossil vertebrates

Athlon: Essays on Palaeontology in Honour of Lovis Shano Russell. Edited by C. S. Churcher. Pp. 286. (Royal Ontario Museum: Toronto, 1976.) \$15.

THIS *Festschrift* volume honours Dr Lovis Russell, who recently retired as Professor of Geology at Toronto University and Chief Biologist at the Royal Ontario Museum. Nearly all the 16 scientific papers are therefore concerned with aspects of North American vertebrate palaeontology, and over half of them discuss vertebrate morphology and systematics. These papers include the following: Dineley, on the mode of life of the heterostracan fish *Ctenaspis*, which seems to have ploughed through the surface layers of sediment; Olson and Lammers on a labyrinthodont amphibian, which seems to be intermediate between the trimerorhachoids and the brachyopoids; Morris, on the hypsilophodont dinosaur *Thescelosaurus*, prefers Thulborn's classification of the group to Galton's; Green and J. E. Martin revise the didelphid marsupial *Peratherium* of the North American Oligocene and Miocene; Turnbull reconstructs the jaw musculature of the South American sabre-toothed marsupial *Thylacosmilus* and shows that the stabbing action was by the action of the hyper-developed ventral cervical musculature, the relative proportions of the jaw-closing muscles not being of specialised carnivore type; Turner and L. D. Martin describe two new rhinocerotoids from the Oligocene of Nebraska; Hooijer compares the pygmy mammoth of the offshore Californian Channel Islands with other dwarf elephants; Lundelius and Slaughter revise the taxonomy of North American Pleistocene tapirs; and A. E. Wood revises the taxonomy of the Oligocene rodent family Ischyromyridae. Finally, at a somewhat higher phyletic level, Carroll redescribes the South African late Permian younginid genus *Heleosaurus*, which shows many similarities to the little early Triassic archosaur *Euparkeria*. He concludes that younginids may be ancestral to the archosaurs, and that the larger early Triassic archosaurs may represent an early divergent specialisation for a semi-aquatic habitat.

Five of the papers are faunal studies. Madeleine Fritz describes an unusual specimen made up of a series of overgrowths of two ectoproct species on a stromatoporoid base. Holman describes Cenezoic herpetofaunas from Saskatchewan and shows that, although the amphibian fauna of the early Oligocene was already essentially modern, the reptile fauna only became modernised

between then and the late Miocene. Wann Langston suggests that the late Cretaceous Scabby Butte fauna was preserved in a marshier environment that lay closer to the sea than the more varied dinosaur faunas of the Edmonton and Oldman deposits. Storer shows that the Hand Hills Formation of Alberta contains both Mio-Pliocene mammals and Pleistocene mammals, the deposit resulting from glacial reworking and mixing. Finally, Hibbard shows that a stratigraphic confusion between three volcanic ash deposits in Kansas has led to the incorrect placing of the Cudahy Pleistocene fauna in the Yarmouthian instead of the Late Kansan.

In the most general (and therefore most generally interesting) paper, Sloan discusses the ecology of dinosaur extinction in western North America as shown by the replacement of the dinosaur-dominated *Triceratops* fauna by the mammal-dominated *Protungulatum-Stygmis* fauna that probably immigrated from Asia. He concludes that

there is now good evidence of a major floral change at this time, there being a 45% drop in floral diversity and a 71% loss of plant taxa by extinction over 1–2 Myr. This change from a subtropical angiosperm-based flora to a cooler, temperate, more conifer-dominated flora was, Sloan suggests, due to climatic changes resulting from the retreat of the North American inland sea from the region.

Most of the papers are above-average in interest, the book is well produced and edited, and there are relatively few typographical errors. Even at prevailing rates of exchange, it is not expensive. As with any such collection, the main problem is that most workers will find that, however good the papers are, only a small proportion are relevant to their particular range of interests.

Barry Cox

Barry Cox is Professor of Zoology at King's College, University of London, UK.

Developmental biology

Explorations in Developmental Biology. By C. Fulton and A. O. Klein. Pp. xv+704. (Harvard University: Cambridge, Massachusetts, 1976.) £11.90.

THE authors of this book state that it is unsatisfactory to teach developmental biology through "formal presentation of a body of knowledge to be passively assimilated by students from books and lectures", and claim a novel approach in providing a collection of original papers, grouped in topics and linked by passages which clarify details, provide questions and suggest further reading. There are 59 papers, giving very broad coverage and dating from Spemann and Mangold (1924); but most of them are post-1960. Despite their praise of original papers and scorn for textbooks, the authors provide two text-type chapters (on amphibian embryology and cells in culture), abridge several papers and give an extract from Twitty's delightful *Of Scientists and Salamanders* rather than the original work.

Do any University teachers instruct in the way criticised by Fulton and Klein? The problem lies in balance. Since research papers are often difficult and time consuming to read critically, it is at least efficient to teach mainly by lectures and texts to elementary students, but to swing later to original sources, when the teacher's role becomes to select reading, help in discussion and provide factual background. The book duplicates this role and, of course, cannot keep up with

the latest information as the teacher can.

A good textbook is not an *ex cathedra* statement of the truth. It stimulates by telling what is known, what is uncertain about what is known, what is unknown and how it might come to be known. As a summary of our knowledge, it can cover immense breadth, and at least mention the oddities. A comparison illustrates this. Fulton and Klein take 63 pages to discuss whether the statement that "genes do not change during embryonic development" is a "cornerstone of developmental biology" or a reasonable working hypothesis. Ebert and Sussex's *Interacting Systems in Development* covers the ground in 5 pages and asks more probing questions. So does Berrill's *Developmental Biology*, and adds the unusual case of *Ascaris*. Fulton and Klein make a hardly fair comparison with a brief and misleading paragraph in an introductory general biology text.

Would anyone find the book useful? Probably not those with access to an adequate library, teaching special aspects in tandem with a team of colleagues. For the teacher struggling to cover the field alone, it may prove a godsend. But a warning: in the areas I know best, the choice of papers and some of the linking commentary is idiosyncratic. This is of course the point about University teaching. It is done by individuals whose choice of emphasis provides an essential diversity. Fulton and Klein's course sounds fun, but it isn't mine.

J. R. Downie

Dr Downie is a Lecturer in Zoology at the University of Glasgow, UK.

Metabolism in filamentous fungi

The Filamentous Fungi. Vol. 2: Biosynthesis and Metabolism. Edited by John E. Smith and David R. Berry. Pp. xiv+520. (Arnold: London, June 1976.) £19.75.

MUCH of the space allocated to this review could be taken up by listing the twenty-two authors and the seventeen chapters which they have contributed to this, the second volume of a series on the filamentous fungi. The writers endeavour to cover all aspects of biosynthesis of carbohydrates, lipids, fatty acids, nucleic acids and proteins and the associated pathways of intermediary metabolism and nutrient assimilation.

The authors, in their attempts to succeed in this, are forced to produce highly condensed articles some of which, although they may be comprehensive, are very hard work to read. Many authors, however, do set out to make their contributions readable and comprehensible to those not working directly in their own field. A. T. Bull and M. E. Bushell begin by providing a good introduction to the problems encountered in the construction and operation of continuous flow fermenter systems for filamentous fungi.

A. H. Rose provides an excellent account of the clinical nature of membrane components. At the other end of the spectrum, one or two authors make few concessions to anyone who is not fully conversant with the terminology and techniques used in their discipline, and consequently have produced chapters which are very difficult to digest.

The latter is not consistent with the editors' intent to include undergraduate and postgraduate students in the readership. Although perhaps not all subjects would have been amenable to this treatment, it would have been useful if the editors had encouraged authors to begin their chapters with a brief introduction to the nomenclature. For example, the chapter on Carotenoids by T. W. Goodwin begins with a good introduction which rapidly accelerates to a detailed exposition with which the reader can keep in step.

An encouraging feature of this book is that a number of chapters conclude with suggestions for useful future developments, whereas volumes presenting up-to-date reviews often leave one with the feeling that most worthwhile work has been done or is in hand. V. W. Cochrane, in the closing paragraphs of his chapter on glycolysis, encourages workers in the field to

investigate *Neurospora crassa* in the same detail with which bacteriologists have studied *Escherichia coli* and also points out that some groups of fungi have been largely neglected. In another instance, J. A. Pateman and J. R. Kinghorn have attempted to present their review of nitrogen metabolism in such a way as "to allow deeper study for those interested in a particular field".

This is a valuable volume as it provides a good review of present knowledge, with copious references, and it may well have the effect of encouraging others to investigate some of the neglected areas of the subject.

H. A. S. Epton

Dr Epton is a Lecturer in Botany at the University of Manchester, UK.

Numerate account of marine ecology

The Ecology of the Seas. Edited by D. H. Cushing and J. J. Walsh. Pp. x+467. (Blackwell: London and Oxford, 1976.) £8.75.

THE names of Cushing, Dugdale, Kelley, Parsons, Steele and Walsh, to mention but 6 of the 14 contributors, should be sufficient to promise good value in this book for marine ecologists. I have no doubt that it is good value, and to be highly recommended.

What these very numerate ecologists aimed to do, under the leadership of Cushing and Walsh, was to set out "the present state of knowledge of the quantitative nature of marine ecosystems": in 6 parts, comprising 16 chapters, with very adequate references and indexes (authors, subjects, species and geographical). They proceed systematically from the characteristics of the seas and the life they maintain, through the forms and functions of marine ecosystems, with some of the consequences in terms of evolution, and of yield for man. The final section is in two parts: the first, an excellent chapter on sampling (by J. C. Kelley), and the second concerning a comprehensive "simulation model" of ecological relationships at three trophic levels, within the upwelling systems off Peru, Baya California and north-west Africa.

In all this, prime emphasis is on the processes concerned, both hydrodynamic and biological: indeed, life in the sea. In association there is emphasis on the absolute necessity for

distinguishing between variability in space and variability in time, which variations have been "confounded" in more senses than one for most aspiring marine ecologists over the past 50 years.

I cannot but be impressed by the way in which so many of the problems—which could be seen only dimly 50 years ago and, although perhaps perceived a little more clearly 25 years ago, still left marine ecologists gravelled for lack of both data and technique—are now (following great developments in both) beginning to yield understanding, and even the possibility of prediction, in such complicated circumstances. That the methodology is far from simple, and the options many, is further illustrated by a quotation from the last chapter, by Walsh (references omitted): "Depending on the problem, one must decide whether steady state or time dependent studies . . . with simulation and/or statistical models . . . involving the analytical or numerical solution . . . of differential or difference equations . . . made of linear or non-linear terms . . . should be solved on analog or digital computers . . . in relation to spatial or single point considerations . . . and exogenous or endogenous driving functions . . . within a stochastic or deterministic parameter space . . .".

As Cushing says in his preface, the contributors are writing as scientists, who have spent long periods at sea, for scientists who are beginning to work at sea. I find it difficult therefore, to think of a better or more challenging introduction. Whether this important science will, however, be able to expand in foreseeable circumstances, in the manner that progress made so far seems to justify, is uncertain to say the least. Quite apart from never-ending arguments about the Law of the Sea, it is inherently very costly, if only in terms of ships and resources required for the extensive synoptic surveys that better understanding demands. For this reason alone, one of the most important points made by Walsh concerns the present challenge to devise new methods of exploiting existing data banks, rather than heedlessly adding to them!

One last point: taking into account the high price and unattractive binding, it is difficult to understand why the publishers let the team get away with duplicating Fig. 7.6 by the identical (except for the legend) Fig. 8.2.

C. E. Lucas

Sir Cyril Lucas was formerly Director of the Marine Laboratory, Aberdeen (retired 1970) and subsequently a member of the Natural Environment Research Council, UK.

announcements

Awards

Alfred Champagnat (France) is the winner of the 1976 Unesco Science Prize, and **Louis Estrada Martinez** (Mexico) and **José Reis** (Brazil) will share the 1974 Kalinga Prize for the popularisation of science.

A prize of DM 10,000 is donated by Boehringer, Mannheim, every two years for outstanding work in the field of biochemical instrumentation and analysis. One or several papers concerning one theme, either published or accepted for publication between October 1, 1975, and September 30, 1977 should be sent to: Prof. Dr I. Trautschold, Medizinische Hochschule Hannover, 3000 Hannover 61, Karl-Wiechert-Allee 9.

The Science Research Council intends to award about fifteen fellowships in 1977 under its new **Advanced Fellowship Scheme**. These fellowships fill the gap between SRC postdoctoral and senior fellowships schemes. They will be awarded to outstanding young workers who have already proved their ability but have not yet obtained a tenured post. The Fellows would have no guarantee of a tenured post on completion of their fellowship although most would be expected to gain such posts in due course. The stipend will be linked to the University lecturer scale. Details of the regulations are available from the Science Research Council, State House, High Holborn, London, WC1. Applications for advanced fellowships to commence on October 1, 1977 should be sent to the Science Research Council by January 31, 1977.

Appointments

Members of the **Genetic Manipulation Advisory Group** have been announced by the Secretary of State for Education and Science. They are Sir Gordon Wolstenholme (Chairman), J. B. Brooksby, K. R. Dumbell, H. J. Evans, J. A. Gilbey, R. J. C. Harris, M. Jahoda, Bernard Langley, J. E. Lawrie, John Maddox, K. Mather, J. Ravetz, M. H. Richmond, J. H. Subak-Sharpe, Sir Brian Windeyer. Further appointments are expected shortly.

Michael John Bevis, Reader in Metallurgy and Materials Science at Liverpool University, has been appointed to

Person to Person

The possibility exists of arranging postdoctoral research in investigations of the kinetics of chemical reactions relevant to terrestrial and planetary environments. Topics currently under study include the kinetics of the formation, decomposition, and photodissociation of mesospheric cluster ions, and the mechanisms of production of interstellar molecules. Informal enquiries should be addressed to R. R. Burke, CRPE, CNET & CNRS, 45045 Orléans Cedex, France.

Echange culturelle. Famille Américaine désire envoyer fils, 17 ans, pour vivre en famille pour une année en France ou en Belgique. En échange nous prendrions le fils ou la fille de la famille hôte et nous l'éleverions comme le nôtre pour une année. Date préférée pour commencer: Juin 1977 (James F. Quinlan, Box 8, Mammoth Cave, Kentucky 42259, United States).

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

the newly created Chair in Non-Metallic Materials at Brunel University. Professor Bevis will become Head of the Department formerly called Polymer Science and Technology.

F. John Bryant of the Department of Physics, Hull, has been appointed to a Personal Professorship.

Reports and Publications

Outside UK—October

US Department of Commerce: National Oceanic and Atmospheric Administration. Marine Geology and Geophysics Data Services and Publications. Pp. 11. (Boulder, Colorado: National Geophysical and Solar-Terrestrial Data Center, 1976.) [2110]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Irrigation Research, 1974-75. Pp. iv + 79. (Griffith, NSW: CSIRO, 1976.) [2110]

Bulletin of the American Museum of Natural History. Vol. 157, Article 4: Shell Ultrastructure of the Atlantidae (Heteropoda, Mesogastropoda) *Oxygyrus* and *Protatlanta*, with Comments on *Atlanta Inclinata*. By Roger L. Batten and Michael P. Dumont. Pp. 263-310. (New York: American Museum of Natural History, 1976.) \$2.90. [2210]

United States Department of the Interior: Geo-

logical Survey. Professional Paper 869: Noncystimorph Colonial Rugose Corals of the Onesquethaw and Lower Cazenovia Stages (Lower and Middle Devonian) in New York and Adjacent Areas. By William A. Oliver, Jr. Pp. v + 156 + 108 plates. Professional Paper 882: Recent Surface Movements in the Baldwin Hills, Los Angeles County, California. By Robert O. Castle and Robert F. Yerkes. Pp. vii + 125 + 4 plates. (Washington, DC: US Government Printing Office, 1976.) [2210]

Smithsonian Contributions to Zoology. No. 230: Contribution to the Polychaete Family Trochochaetidae Pettibone. By Marian H. Pettibone. Pp. 21. No. 231: Benthic Marine Cypridinacea from Hawaii (Ostracoda). By Louis S. Kornicker. Pp. iii + 24. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [2510]

Chemical Evolution: Comparative Planetology. (The Third College Park Colloquium on Chemical Evolution, September 29-October 1, 1976.) Pp. 57. (College Park, Maryland: Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, 1976.) [2510]

The Phaistos Disc: An Interpretation of Astronomical Symbols. By Leon Pomerance. Pp. 76. (Göteborg: Paul Aströms Förlag, 1976.) [2510]

CERN—European Organization for Nuclear Research. CERN 76-15: Electronic Equipment for Wire-Stretching Machines. By N. Rasmussen. Pp. vii + 15. (Geneva: CERN, 1976.) [2510]

United States Department of the Interior: Geological Survey. Professional Paper 929: ERTS-1: a New Window on Our Planet. Edited by Richard S. Williams, Jr., and William D. Carter. Pp. xix + 362. (Washington, DC: US Government Printing Office, 1976.) [2510]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2131: Surface Water Supply of the United States, 1966-70. Part II: Pacific Slope Basins in California. Vol. 4: Northern Central Valley Basins. Pp. ix + 747. (Washington, DC: US Government Printing Office, 1976.) [2610]

Reactor Centrum Nederland. Summary of Activities July 1975-July 1976. Pp. 20. ('s-Gravenhage: Reactor Centrum Nederland, 1976.) [2710]

Ozone Depletion in the Atmosphere: an Annotated List of References. (Library Bibliography Series No. 3). Pp. 24. (Orlando, Florida: Florida Technological University, 1976.) [2710]

Eine neue Theorie über die Gravitation aus Kybner-netischer Sicht. Von Johan C. Metz. Pp. 26. (Aix-la-Chapelle: Johan C. Metz, Hüttenstr. 66, 1976.) [2710]

Annals of the Transvaal Museum. Vol. 30, No. 7: The Tenebrionidae of Southern Africa, XLIV. A New *Gonopus* (*Agonopus*) Species from the Transvaal (Coleoptera). By L. Schulze. Pp. 91-95 + plate 9. Vol. 30, No. 8: On a Newly Associated Composite Mandible from Swartkrans (Mammalia: Hominidae). By T. D. White. Pp. 97-98 + plates 10 and 11. Vol. 30, No. 9: Trap Response of some Southern African Small Mammals. By J. A. J. Nel and I. L. Rautenbach. Pp. 99-104. Vol. 30, No. 10: The Type-Material of the Nominal Species *Charaxites vansoni* Van Someren (Lepidoptera: Nymphalidae). By R. I. Vane-Wright. Pp. 105-107. (Pretoria: Transvaal Museum, 1976.) [2710]

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newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. There is a Reader Enquiry Form on the page facing the inside back cover.

Automated X-ray powder diffractometer. Philips. The APD 10 comprises a Philips 3 kW generator with vertical goniometer equipped with graphite monochromator and 35-sample changer, plus associated controls and chart recorder; the additional automation facilities are controlled by a mini-computer using plain language instructions via a teletypewriter. A dual station cassette unit enables measurement data to be collected for off-line processing on a larger computer. Standard programs are available. The APD 10 enables routine work to be done overnight or at weekends. Applications cover geology, mining, steel production, petro-chemicals, paint and air pollution.

Circle No. 43 on Reader Enquiry Form

Double beam atomic absorption spectrophotometer. Pye Unicam Ltd. The SP2900 has both peak height and peak area measurement which can be remotely initiated. The timing for peak height measurement is from 0-99.9 seconds in 100 msecond steps and peak area integration is over a 4 or 10 second period. The built-in lamp turret keeps four pre-focussed lamps aligned, powered and ready to work. Lamp selection is optical. The SP2900 has aircraft-style, 7-bar, bi-polar illuminated digits. The range is -0.05 to $1.999A$ with a switchable decimal point and double over-range indication. Readout is in absorbance, concentration or emission units and digital update is $2.5\times$ every second. A concentration calculator helps produce fast and accurate results with a single operation of curvator and scale expansion controls using just two standard solutions. The single curvature control is independent, wide-ranging and does not affect zero. Calibrated scale expansion is from $0.2\times$ to $50\times$ (analogue and digital). The background correction accessory provides automatic, simultaneous correction for high background absorbances—particularly important when using electro-thermal atomisation techniques. The SP2900 corrects for 1.5A of background to better than 1%.

Circle No. 44 on Reader Enquiry Form

Linear displacement monitor. Rofin Ltd. High resolution dual or single axis light spot position sensing system includes one- or two-axis position resolution to 0.0001 inch, independent of zero and sensitivity control for each axis, analogue output with frequency response to 3KHz and position sensitivity independent of light spot size when used with a Helium-Neon or other visible C.W. laser. Position sensitivity is dependent on the detector type used, incident light power and the sensitivity setting. Applications include: flatness . . . positioned over a surface, the monitor gives a meter indication of the deviation from flatness at any point; auto-collimating . . . using spherically corrected lenses, the displacement display can measure pitch and roll angles; motion monitoring . . . with the high frequency analogue output and linearity of output, the monitor can measure vibration rate and amplitude.

Circle No. 45 on Reader Enquiry Form

Immersible molecular separator. Millipore. Sized to handle volumes between 1 and 100 ml, the separator is ideal for concentrating proteins, viruses and colloids, removing salts, buffers and



Immersible molecular separator

solvents, deproteinising samples and separating unbound ligands. The unit can be reused after a simple cleaning operation. The device is lowered into the solution to be processed and is then connected to any convenient vacuum source. Filtrate passes through the membrane into the core, and then flows via the vacuum line to a suitable trap. Retained molecules (the retentate) remain in the original sample container. Both the retentate and the filtrate fractions can be recovered. Filtration can be automatically terminated at any preselected point.

Circle No. 46 on Reader Enquiry Form

Amino acid analyser. Mark Instrument Company. The newly-developed system provides single column analysis for protein hydrolyzates and physiological fluids. The Model AAA-F-100 Amino Acid Analyser eliminates lengthy and laborious manual analyses and affords the following benefits: complete and permanent strip chart presentation of the amino-acid constituents of the sample, the quantity and sequence of their elution from analyser's ion exchange column; only a minute sample is required; more accurate data and reproducible results; flexibility through easy modification, addition of accessories, or variations in operational conditions; table top size.

Circle No. 47 on Reader Enquiry Form

Multi-point recording facility. Chandos. A new electronic attachment provides multi-point recording facilities from a single channel recorder. Two or more of these units could be used with existing dual channel or multi-channel recorders if they are continuous trace. The system provides a channel identification datum and a means of adjusting the time spent recording individual points. Models accommodating up to 36 points are available for various types of measurement—pressure, temperature, pH, conductivity, oxygen, selective ions etc. Adjustment and zero suppression can be built in to provide a given full scale deflection.

Circle No. 48 on Reader Enquiry Form

Miniature accelerometer. SE Labs. The EGAI. 125-5, measures acceleration, vibration and shock from 0.001g to 5g. Weighing less than 0.5 g and occupying less than 0.006 cubic inches, the EGAI is ideal for situations requiring minimal weight addition to the item under test. Applications vary from tremor studies on human and animal subjects to vibration and flutter analysis on vehicle models undergoing wind tunnel tests. The EGAI is a semi-conductor strain gauge device which measures static and dynamic acceleration with a sensitivity of 15mV/g unamplified. Available with six different mounting facilities, it has an operational temperature range of -40 to $121^{\circ}C$. In addition, it is available with a 0.7 critical damping option.

Circle No. 49 on Reader Enquiry Form